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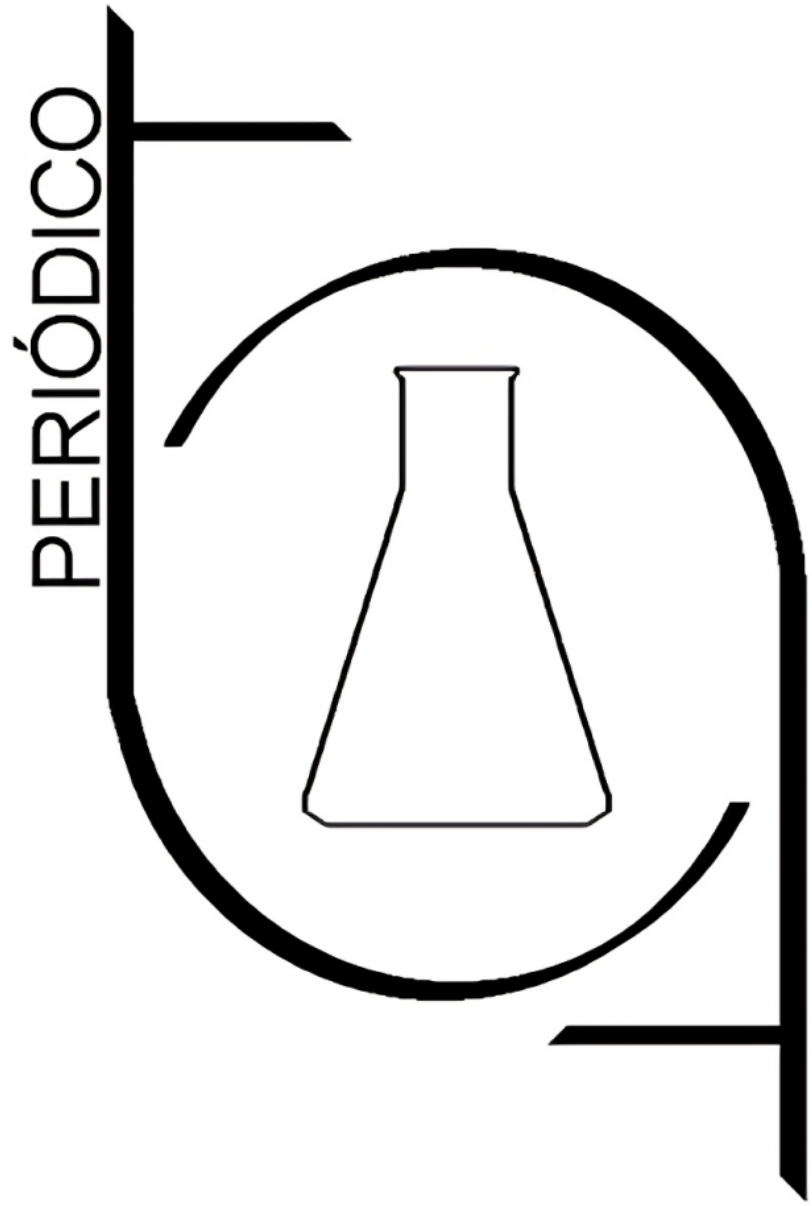

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SIMULAÇÃO INTERATIVA BASEADA EM INTELIGÊNCIA ARTIFICIAL PARA MODELOS ATÔMICOS: ESTUDO PILOTO

AI-BASED INTERACTIVE SIMULATION FOR ATOMIC MODELS: PILOT STUDY

ATOM MODELLERİ İÇİN YAPAY ZEKA TABANLI ETKİLEŞİMLİ SIMÜLASYON: PİLOT ÇALIŞMA

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RESUMO

Introdução: Os modelos atômicos são ensinados de forma abstrata e mecânica, o que limita a compreensão conceitual e as habilidades de raciocínio dos alunos. Este estudo aborda esses desafios por meio de uma simulação interativa baseada em IA que integra o desenvolvimento histórico dos modelos atômicos em uma estrutura tecnológica e pedagógica por meio de diálogos científicos, promovendo a investigação, a participação e a aprendizagem significativa no ensino de química. **Objetivo:** Este estudo tem como objetivo avaliar a eficácia da simulação baseada em IA no desenvolvimento da compreensão conceitual, participação e habilidades de raciocínio científico dos alunos do ensino médio em relação aos modelos atômicos. **Métodos:** O estudo utilizou o método de pesquisa de design e desenvolvimento. A simulação foi criada na plataforma Replit usando a abordagem de diálogo científico suportada pelo GPT-4.0. Cinquenta alunos do 9º ano participaram voluntariamente do estudo. Os dados foram coletados por meio de perguntas abertas e analisados usando escalas de usabilidade, experiência do usuário e carga cognitiva. **Resultados e Discussão:** Os alunos classificaram a simulação como tendo alta usabilidade (M=4,07), boa experiência do usuário (M=3,76) e baixa carga cognitiva (M=2,36). Os comentários qualitativos destacaram que os diálogos científicos eram agradáveis e fáceis de usar. Os resultados mostram que a simulação aumentou efetivamente a compreensão e a participação dos alunos na aprendizagem dos modelos atômicos. O estudo é consistente com trabalhos anteriores que enfatizam o papel das simulações digitais no desenvolvimento da aprendizagem conceitual. O feedback apoiado por IA e os diálogos interativos promoveram a motivação e o raciocínio científico. **Conclusões:** A simulação aumenta a motivação dos alunos e a compreensão conceitual ao integrar elementos históricos, pedagógicos e tecnológicos. Ela apoia a aprendizagem significativa e pode ser expandida com aplicações mais amplas em estudos futuros.

Palavras-chave: *Educação química, aprendizagem baseada em cenários, tecnologia educacional, compreensão conceitual*

ABSTRACT

Background: Atomic models are typically taught using abstract and rote methods, which limit students' conceptual understanding and reasoning skills. This study addresses these challenges through an AI-based interactive simulation that integrates the historical development of atomic models within a technological and

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pedagogical framework via scientific dialogues, promoting inquiry, participation, and meaningful learning in chemistry education. **Aims:** This study aims to evaluate the effectiveness of AI-based simulation in developing high school students' conceptual understanding, participation, and scientific reasoning skills regarding atomic models. **Methods:** The study utilized the design and development research method. The simulation was created on the Replit platform using a scientific dialogue approach supported by GPT-4.0. Fifty 9th-grade students volunteered for the study. Data were collected through open-ended questions and analyzed quantitatively and qualitatively using usability, user experience, and cognitive load scales. **Results:** Students rated the simulation as having high usability (M=4.07), good user experience (M=3.76), and low cognitive load (M=2.36). Qualitative feedback emphasized that the scientific dialogues were enjoyable and easy to use. Findings showed increased participation, improved conceptual understanding, and positive learning experiences. **Discussion:** Results suggest that the simulation effectively increased students' understanding and engagement in learning atomic models. Findings are consistent with previous studies highlighting the role of digital simulations in enhancing conceptual learning. AI-supported feedback and interactive dialogues encouraged motivation and scientific reasoning. **Conclusions:** Simulation increases student motivation and conceptual understanding by integrating historical, pedagogical, and technological elements. It supports meaningful learning and can be expanded with broader applications in future studies.

Keywords: *Chemistry education, scenario-based learning, educational technology, conceptual understanding*

ÖZET

Arka plan: Atom modelleri genellikle soyut ve ezberci yöntemlerle öğretilir, bu da öğrencilerin kavramsal anlayış ve bilimsel akıl yürütme becerilerini sınırlar. Bu çalışma, bilimsel diyaloglar yoluyla atom modellerinin tarihsel gelişimini teknolojik ve pedagojik bir çerçeveye entegre eden, kimya eğitiminde sorgulama, katılım ve anlamlı öğrenmeyi teşvik eden yapay zekâ tabanlı etkileşimli bir simülasyon aracılığıyla bu zorlukları ele almaktadır. **Amaçlar:** Bu çalışma, lise öğrencilerinin atom modelleriyle ilgili kavramsal anlayış, katılım ve bilimsel muhakeme becerilerini geliştirmede yapay zekâ tabanlı simülasyonun etkinliğini değerlendirmeyi amaçlamaktadır. **Yöntemler:** Çalışmada tasarım ve geliştirme araştırma yöntemi kullanılmıştır. Simülasyon, GPT-4.0 tarafından desteklenen bilimsel diyalog yaklaşımı kullanılarak Replit platformunda oluşturulmuştur. Çalışmaya toplam 50 9. sınıf öğrencisi gönüllü olarak katılmıştır. Veriler açık uçlu sorularla toplanmış ve kullanılabilirlik, kullanıcı deneyimi ve bilişsel yük ölçekleri kullanılarak nicel ve nitel olarak analiz edilmiştir. Sonuçlar: Öğrenciler simülasyonu yüksek kullanılabilirlik (M=4,07), iyi kullanıcı deneyimi (M=3,76) ve düşük bilişsel yük (M=2,36) olarak değerlendirmiştir. Niteliksel geri bildirimler, bilimsel diyalogların eğlenceli ve kullanımı kolay olduğunu vurguladı. Bulgular, katılımın arttığını, kavramsal anlayışın geliştiğini ve olumlu öğrenme deneyimleri yaşandığını gösterdi. **Tartışma:** Sonuçlar, simülasyonun öğrencilerin atom modellerini öğrenme konusundaki anlayışlarını ve katılımlarını etkili bir şekilde artırdığını göstermektedir. Bulgular, kavramsal öğrenmeyi geliştirmede dijital simülasyonların rolünü vurgulayan önceki çalışmalarla tutarlıdır. AI destekli geri bildirimler ve etkileşimli diyaloglar, motivasyonu ve bilimsel akıl yürütmeyi teşvik ettiği görülmüştür. **Sonuçlar:** Simülasyon, tarihsel, pedagojik ve teknolojik unsurları entegre ederek öğrencilerin motivasyonunu ve kavramsal anlayışını artırmaktadır. Anlamlı öğrenmeyi desteklemekte ve gelecekteki çalışmalarda daha geniş uygulamalarla genişletilebilir.

Anahtar Kelimeler: *Kimya eğitimi, senaryo temelli öğrenme, eğitim teknolojisi, kavramsal anlama*

1. INTRODUCTION:

The atom is one of the most important ideas in science, and students need to comprehend it correctly for many fields, including chemistry, physics, and biology. Atomic models are commonly taught in an abstract, rote way in high school chemistry classes, which makes it hard for students to really understand the concepts and leads to many misconceptions (Malkawi *et al*., 2018; Zarkadis, Stamovlasis & Papageorgiou, 2020; Kaya, 2023). Scientific models connect theories to the real world, making abstract ideas more concrete. However, if this process isn't adequately organized, students' understanding of concepts stays limited (Justi & Gilbert, 2000; Mansoor & Rodríguez, 2001; Niaz *et al*., 2002).

The lack of attention to historical and scientific backgrounds, especially in atomic model teaching, is regarded as a major deficiency (Weisi & Zamani, 2015; Cardoso *et al*., 2020). Currently, interactive computer-based simulations allow students to explore abstract ideas, compare different models, and simulate scientific reasoning procedures (Fabrigas & Paglinawan, 2024; Kefalis, Skordoulis & Drigas, 2025). In particular, PhET Interactive Simulations have been known to promote students' modeling aptitudes and critical comparisons (Wieman, Adams & Perkins, 2008; Moore *et al*., 2014; Haryadi & Pujiastuti, 2020).

Furthermore, recent discussions regarding the digital transformation of chemistry education emphasize the necessity of technology-supported, sustainable, and pedagogically consistent learning environments (Köfter, Altundağ & Yücel, 2025). In line with this, recent applications of artificial intelligence as well as machine learning in teaching chemistry have great potential to renovate learning environments by providing benefits including, but not limited to, personalized learning, real-time feedback, as well as dynamic content development (Iyamuremye *et al*., 2024; Maity & Deroy, 2024; Wang *et al*., 2024; Erümit & Sarıalioğlu, 2025). This research work aims to help students learn and understand atom models through an interrogative approach, drawing on historical and scientific backgrounds. The study seeks to link scientific teaching principles from higher education with learning psychology to help students build concepts (Nadelson *et al*., 2018; Valeeva *et al*., 2023). The AI-supported learning environment developed in this direction is designed to holistically support students' conceptual comparison, critical thinking, and historical connectivity skills. The conceptual test and personalized feedback mechanism at the end of the application support the measurement and evaluation process, thereby deepening learning.

This study aims to investigate the integration of AI-supported interactive simulation into the teaching of atomic models, grounded in a historical context. The following questions guide the research:

- How can an AI-supported interactive simulation centered on historical context be adapted to the teaching of atomic models?
- How does such a simulation affect students' conceptual understanding of atomic models?
- How do students rate the usability and level of interaction of the simulation?
- What are the student evaluations regarding the simulation's contribution to the learning process?

The initial design of this study was presented orally at the ICETOL Conference held in Cunda, Ayvalık, on August 26–29, 2025. Based on feedback received at the conference, the simulation was restructured, and the prototype was then tested in a pilot application with high school students.

2. MATERIALS AND METHODS:

The methodology of this study was designed to systematically develop, implement, and evaluate an artificial intelligence-based simulation to help students better understand atomic models. The following sections detail the participants, the study design, the development process, and the data collection procedures.

2.1. Participants

The study was conducted with 50 9th-grade students enrolled in public high schools in Ankara, Turkey, during the 2024–2025 academic year. Participation in the study was voluntary, and parental consent was obtained prior to the implementation.

The participants had not yet received formal instruction on atomic theories at the time of the study. The simulation was implemented immediately before the curriculum unit on atomic models to support students' initial conceptual engagement with the topic.

Only students with access to a digital device and the basic skills required to interact with a web-based simulation environment were included. Students who had previously studied atomic theory or did not complete the simulation were excluded from the study. To protect participant privacy, all data were collected anonymously, and no personally identifiable information was recorded.

2.2. Study Design

This paper is structured within the Design and Development Research framework (Richey & Klein, 2014). In this study, an artificial intelligence-based simulation environment was designed and developed to support the teaching of atomic models in a historical and interactive context, and its effectiveness was evaluated by using it to high school students.

2.3. Development Process and Implementation

The development of the simulation followed a structured instructional design sequence consistent with widely accepted frameworks for multimedia learning environments, particularly the ADDIE model (Analysis, Design, Development, Implementation, Evaluation) (Göçer, Mustafaoğlu, & Alkan, 2026). The design process proceeded through the phases below:

1. Needs and Context Analysis: An examination of high school students' conceptual learning challenges related to atomic models was conducted, informed by existing literature; a concept map was developed based on the Turkish Ministry of National Education's 9th-grade chemistry curriculum (Ministry of National Education [MoNE], 2024).

2. Scenario Development: Original conversations were produced between six scientists from the curriculum (Dalton, Thomson, Rutherford, Bohr, Chadwick, and Heisenberg) while keeping in mind historical authenticity, humorous moments, and curriculum objectives.

3. Digitization and AI Integration: The scenario was turned into an interactive app on the Replit platform. The scientist figure used GPT-4.0-based AI to answer the students' questions.

4. Interface Design: A web interface was created that is easy to use and understand.

5. Assessment Module: At the end of the simulation, there was a test with 10 multiple-choice questions. For each question and the overall assessment, the scientist character provided tailored feedback using artificial intelligence.

2.3.1. Needs and Context Analysis

The needs and context study examined the 9th-grade chemistry curriculum issued by the Ministry of National Education of the Republic of Turkey. It identified essential concepts derived from this initiative (MoNE, 2024). The identified topics include aspects of scientific discourse, such as atomic structure, the historical development of atomic models, the experimental data validating these models, and the differing perspectives among scientists. A concept map appropriate for the students' level of conceptual development was developed (Figure 1).

The developed concept map served as the foundation for organizing the simulation content, crafting dialogue scenarios, and establishing evaluation criteria. This analytical procedure was deemed a crucial preparatory step for maintaining pedagogical integrity and creating innovative digital teaching materials aligned with the curriculum.

2.3.2. Scenario and Dialogue Development

The basic framework of this simulation is a dialogue among six major scientists (Dalton, Thomson, Rutherford, Bohr, Chadwick, and Heisenberg) who contributed to the modeling of atoms in the past centuries. This scenario is designed to maintain scientific accuracy and educational effectiveness. Jokes, references, and scientific explanations have been evenly incorporated into these dialogues to interest students, provide them with useful context for concepts, and raise their awareness of scientific thinking processes.

The excerpt below is an example of the distinct writing style as well as scientific debate structure for the characters in the simulation:

- Heisenberg: “Are you still sure where the electron is? I’m not. My uncertainty principle says you can’t know both its location and its speed at the same time.”
- Bohr: “Your ideas have disrupted my orbits. But I admit, the orbital concept is much more accurate.”
- Thomson: “If I hadn’t discovered the electron, none of you would be here. It all started with my plum pudding model!”
- Rutherford: “You talk a good game, Thomson, but I shattered your cake! I found the nucleus with my gold foil experiment.”
- Bohr: “You both did great work, but I brought order to that nucleus. I arranged the electrons into energy levels!”
- Heisenberg: “Did you say order, Bohr? Electrons don’t like discipline. In my world, they’re all a bit lazy and uncertain.”
- Dalton: “You’ve messed up the atom! In my time, everything was simple, clear, and measurable.”
- Chadwick: “Gentlemen, please... Silence. Neutrons don’t talk, but they get the job done.”

In such statements, scientists both argue for their own models and take a critical approach to each other's contributions, thereby revealing the nature of scientific progress. The dialogue framework incorporates features such as intellectual conflict, contrast, and historical context.

These types of discussions enable students to view scientific models not merely as units of knowledge to be memorized, but as systems of ideas that change and evolve scientifically over time and connect with other ideas. In this way, students develop critical thinking, questioning, and conceptual connection skills as they construct scientific models.

2.3.3. Technical Implementation and Evaluation Process of the Simulation

The scripted dialogue scenario was transformed into an interactive simulation environment through an AI-powered development process on the Replit platform. This web-based digital learning environment, called “AtomVerse,” combines historically accurate text-based scenarios with character representations, user interactions, and AI-based feedback (Figure 2).

The application is based on system commands modeled using the OpenAI GPT-4.0 model. These system commands have a multi-

layered structure that defines each scientist's style, scientific approach, and interaction method. A total of 11 different GPT-4.0 commands are used in the application, and these commands are organized into three major categories:

1. Generating Answers to Students' Questions: Ensures that scientist characters provide contextually and scientifically consistent answers to questions asked by students during discussions.
2. Test Feedback System: At the end of the simulation, a 10-question multiple-choice test is presented, and for each question, the relevant scientist character provides explanatory and educational feedback.
3. Performance Assessment: The students' overall success, strengths, and areas for improvement are summarized by the analysis module and converted into a personalized final report.

This structure enables students to take on the role of an interactive and curious user who actively participates in their own learning process, rather than merely being an observer (Figure 3). While observing dialogues between scientists, students could ask questions about concepts they want to learn about, and these questions are answered in a scientific manner.

The Graphical User Interface (GUI) was developed on React 18, TypeScript, and Tailwind CSS technologies. During the design process, user experience was prioritized, resulting in a simple, historically aesthetic, and mobile-friendly interface supported by text bubbles, character icons, information cards, and control buttons. Replit's web infrastructure enables this application's online accessibility.

The test at the end of the simulation assesses students' conceptual understanding and guides this process using GPT-4.0-powered explanatory feedback (Figure 4).

Finally, after the test is completed, the system performs data analysis of student performance and generates a personalized final assessment containing a summary of students' achievements and tips for improvement (Figure 5).

Students accessed the application via a web-based interface and participated in the scientists' interactive discussions. In this way, AtomVerse blends historical, pedagogical, and technological features to offer students an innovative teaching solution that integrates artificial intelligence into chemistry education.

2.4. Data Collection

A mixed-methods approach was employed to collect data after students engaged in the AtomVerse simulation. The application lasted approximately 20 minutes in total, during which time students accessed the simulation individually in the computer lab. Finally, after completing the simulation, students were asked to complete the AtomVerse Student Experience Survey, which aimed to measure usability, user experience, and cognitive load. The survey included a mixed-methods design comprising five open-ended questions and eleven Likert items, using the UMUX-Lite, UEQ-S, and Cognitive Load Rating Scale tools.

2.5. Data Analysis

Data collected from the AtomVerse Student Experience Survey was analyzed using both quantitative and qualitative approaches. Quantitative data was processed using IBM SPSS Statistics 26 software to calculate descriptive statistics such as the mean and standard deviation for each dimension of the scale. The internal consistency of the instrument was tested using Cronbach's alpha coefficient, and the normality of the data distribution was checked using skewness and kurtosis values. Repeated measures ANOVA analysis was also used to investigate possible differences between usability, user experience, and cognitive load scales. Qualitative data collected from the five open-ended questions were analyzed using thematic content analysis. Responses were coded inductively to identify recurring themes related to students' learning experiences, perceptions, and suggestions. All analyses were conducted systematically to ensure the validity and reliability of the findings. For open-ended questions, data analysis used thematic content analysis to examine respondents' answers.

3. RESULTS AND DISCUSSION:

3.1. Results

The results of the Student Experience Survey are presented using both quantitative and qualitative criteria. The results reveal how the

simulation was evaluated in terms of usability, user experience, and cognitive load; they also reflect students' overall perceptions and suggestions thematically.

3.1.1. Quantitative Findings

The survey data were collected from 50 9th-grade students. The scale is a 5-point Likert-type scale and consists of 11 items. Three sub-dimensions were evaluated. Representative sample items from each sub-dimension are provided below:

- Usability (UMUX-Lite): "The application meets my needs. '' / "It was easy to use. "
- User Experience (UEQ-S): "The application was fun." / "It looked good. "/ "It was motivating."
- Cognitive Load: "The application was mentally exhausting. '' (negative item)

According to the analysis results, the reliability coefficient of the scale was found to be Cronbach's α=0.73; this value indicates that the scale has an acceptable level of internal consistency.

Repeated Measures ANOVA results revealed a significant difference among dimensions, $F(2, 98) = 63.48$, $p < .001$. Students' perceptions of AtomVerse are ranked by average score in Table 1.

These results indicate that students generally evaluated the application as an easy, motivating, and supportive learning environment. In particular, the items "Easy to use" ($\bar{x}$= 4.22) and "I found it generally good" ($\bar{x}$= 4.22) received the highest scores. In contrast, the items "It was creative" ($\bar{x}$= = 3.12) and "It was interesting" ($\bar{x}$ = 3.46) received relatively lower scores, indicating that the depth of interaction could be increased by diversifying the application's content.

Table 1. *Dimension-Based Average Results for the AtomVerse Application*

Dimension	Mean ($\bar{x}$)	Std. Dev.	Comment
Usability	4.07	0.85	The application is user-friendly and easy to find.
User Experience	3.76	0.66	Aesthetic, motivational, and entertainment elements are strong.
Cognitive Load	2.36	1.06	The application was not found to be mentally taxing.

3.1.2. Quantitative Findings

A content analysis of the five open-ended questions in the AtomVerse Student Experience Survey was conducted, and codes were created based on the themes. The codes for each question were supported by sample statements taken directly from the students' opinions.

Question 1: "What did you like most about using the AtomVerse application?" Two main codes emerged from this question: Scientific Dialogue and Interaction. The statements belonging to the scientific dialogue code are as follows: "I liked the scientists bantering with each other" (S12), "Scientists talking among themselves" (S24). The statements related to the Interaction code are as follows: "I liked that it answered my questions" (S32), "Being able to ask questions" (S37).

Students particularly liked the dialogues and discussions between scientists. The expressions "asking questions" and "getting answers" were frequently repeated. This shows that students perceived the interactive and humorous learning environment positively.

Question 2: "Was there any point where you struggled while using the application?" According to the content analysis, a single code emerged from this question: Ease of Use. Statements related to the easy-to-use code: "The messages came one after another, so I couldn't understand them easily" (S1), "The application was easy" (S15), "It was very easy and explanatory" (S28).

The vast majority of students stated that they did not experience any difficulties. Although some students noted that the messages passed quickly, the general opinion is that the application

is easy to understand.

Question 3: “How did this application help you learn atomic models?” According to the content analysis of this question, two codes were identified: Retention and Fun Learning. Statements belonging to the Retention code: “It opens your mind and becomes lasting” (S7), “It made it more memorable” (S26), “I understood the topic while Dalton and his friends were discussing it” (S36). Expressions belonging to the Fun Learning code: “It was fun because it was like a game” (S7), “It explains things in a fun way” (S34), “Both fun and educational” (S30).

The majority of students directly used the expression “it was helpful.” The prominence of the “‘lasting learning” and “fun learning” codes supports the idea that the application functions as a tool that facilitates learning.

Question 4: “What was the difference compared to regular classes?” Two codes emerged from this question: Active Learning and Attractiveness. Statements related to the Active Learning code: “It has an educational artificial intelligence” (S10), “It was different and nice because it was both technological and answered my questions” (S18), “The environment was comfortable because it could be done outside of school” (S36). Expressions related to the attention-grabbing code: “It was nicer because it was in a digital environment’‘(S41), “It created a nice difference” (S49), “It was both different and made learning easier” (S46).

Students described the application as “more fun,” “more attention-grabbing,” and “better.” These findings show that AtomVerse increases active participation and motivation, unlike traditional lessons.

Question 5: “What changes would you suggest improving the application?’ Two codes emerged from the content analysis: Audio Narration and Visual-Auditory Enrichment. Statements related to the audio narration code: “I would like audio to be added” (S5), “Audio could be added, AI could speak in a more human-like way and be a little more argumentative when discussing” (S11), “There could be a read-aloud feature and slower messaging” (S24). Expressions related to the visual-audio enrichment code: “The text could be larger and faster’‘(S22), “There could be an impressive background” (S28), “The background is overwhelming, it could be changed” (S35).

The most frequently mentioned suggestion was to add a “voice feature” to the application. Some students also suggested adding new features or diversifying response formats. Overall, students are satisfied with the application, and their suggestions are focused on improvement.

3.2. Discussions

The AtomVerse simulation illustrates an original methodology that integrates historical, pedagogical, and technological consistency in the instruction of atomic models. Presenting scientific concepts within a historical timeline and addressing them comparatively supports students’ ability to establish conceptual integrity and develop a consistent mental model of atomic theory.

The representation of scientists using the language specific to their own era exemplifies the adaptation of the scenario-based learning approach (Schank *et al.*,1994) and the theory of concept development through sociocultural interaction (Vygotsky & Cole, 1978) to a digital learning environment. This structure facilitates the transformation of abstract scientific concepts into concrete experiences, enabling students to construct meaning through active interaction and reflection.

Consistent with previous work in the field (Fabrigas & Paglinawan, 2024; Kefalis, Skordoulis, & Drigas, 2025), digital simulations have been shown to facilitate the understanding of complex and abstract concepts. In this regard, AtomVerse provides a personalized and interactive learning experience for students. Furthermore, an entertaining depiction of scientists helps students become emotionally engaged in the learning process, whereas scientific dialogues in an interactive test module help foster students' scientific argumentation skills.

Finally, the performance analysis using AI in this simulation helps students know their areas of proficiency as well as those that require improvement, contributing to students’ self-awareness and self-regulation skills. Thus, AtomVerse goes beyond being a mere information-transferring system to offer an interactive learning environment where the student is an active part of their own learning process.

4. CONCLUSIONS:

This study creates, implements, and develops AtomVerse, an interactive simulation utilizing artificial intelligence technologies, thereby offering a new solution that provides a coherent combination of historical, pedagogical, and technological consistency in the teaching of atomic models.

Quantitative findings from the implementation process show that students rated AtomVerse as extremely user-friendly (average usability score: 4.07 ± 0.85), motivating, and cognitively non-challenging (low cognitive load: 2.36 ± 1.06). These results highlight the simulation's accessible and functional structure, enabling smooth navigation without mental exhaustion. Qualitative insights reveal that students particularly value interactive elements such as "scientific dialogues" and "opportunities to ask questions," describing this experience as more engaging, dynamic, and enjoyable compared to traditional lessons. This feedback highlights AtomVerse's role in promoting meaningful learning in both cognitive and sensory dimensions and is consistent with previous research on digital simulations in chemistry education (Fabrigas & Paglinawan, 2024; Kefalis, Skordoulis, & Drigas, 2025).

As detailed in the discussion, AtomVerse goes beyond mere knowledge transfer by providing an interactive environment where students actively construct knowledge. The scientists' dialogues exemplify the adaptation of scenario-based learning (Schank *et al.*, 1994) and Vygotsky's sociocultural interaction theory (Vygotsky & Cole, 1978) to the digital context, transforming abstract, atomic concepts into concrete, reflective experiences. From a technological standpoint, Replit is the platform used to create this simulation, relying on a web-based integrated development environment (IDE) for collaborative coding, real-time preview, and API connectivity to artificial intelligence systems for data analysis, after consideration of other platforms including Glitch, CodeSandbox, Firebase/Vercel, and Cursor, which were eliminated for Replit's ease of accessibility, development speed, multiple-user capabilities, as well as completely web-intuitive deployment capabilities regardless of device types for more sustainable purposes in different contexts of educational applications.

Consequently, AtomVerse distinguishes itself as a singular simulation that seamlessly integrates historical background, comedic features, and AI-driven personalized feedback, setting it apart from conventional tools such as PhET, ChemCollective, and Labster through its cohesive content delivery approach. While PhET primarily focuses on interactive visualizations, Labster emphasizes virtual laboratory experiments. AtomVerse, on the other hand, offers a narrative-driven, dialogue-based interaction model that contextualizes scientific knowledge within its historical development. It effectively enhances the conceptual understanding of atomic models, increases learning motivation, and develops scientific argumentation skills, demonstrating the transformative potential of AI-focused simulations in chemistry education.

However, limitations remain, particularly regarding the accuracy of GPT-4.0 responses, which depend on the quality of the command. To mitigate this, auditable feedback mechanisms are recommended to enhance system reliability. These mechanisms may include logging responses, automatic fact-checking layers (e.g., cross-verification with reliable databases), human-supervised hybrid models, or user feedback loops; thus, consistency is enhanced through prompt optimization (e.g., using standard prompt structures like ROT), while the pedagogical integrity of educational outputs is preserved. Future work could further strengthen the reliability of AI-based tools by integrating these approaches. In the future, AtomVerse, with its student-centered, interaction-focused, and pedagogically sound structure, promises broader integration into educational ecosystems. Future adaptations may include audio narration, audiovisual enhancements, language options, and applications for various age groups, further increasing its inclusivity and impact.

5. DECLARATIONS

5.1. Study Limitations

Several limitations should be considered when interpreting the findings of this study. The research adopted a design-and-development methodology; therefore, the findings primarily reflect the pedagogical potential, practicality, and applicability of the AtomVerse simulation rather than its causal effects on students' learning outcomes. Consequently, conclusions regarding instructional effectiveness should be interpreted within the boundaries of exploratory and developmental research.

The study involved a sample of 50 ninth-grade students from public high schools in a single

urban location, using convenience sampling. The small sample size and non-random sampling method limit the applicability of the findings to broader student populations, diverse school types, and varying socio-educational contexts.

Resource and equipment constraints also need to be acknowledged. Effective use of the AtomVerse simulation requires access to digital devices and a stable internet connection, which may not be equally available across all educational settings. In addition, the effectiveness of the AI-supported feedback mechanism depends on prompt design quality and the response-generation characteristics of the GPT-4.0 model, which may influence the consistency, depth, and precision of the feedback provided to students.

This study, which focuses mostly on atomic models, is especially pertinent to high school chemistry classes. As a result, even though the findings hold true for comparable grade levels and learning resources, pedagogical, contextual, and content-specific modifications may be necessary to make them applicable to different academic fields or educational phases.

The study confines its focus to the design of the AtomVerse simulation and its preliminary implementation in the classroom. This study does not consider long-term learning outcomes, knowledge retention, or comparative effectiveness evaluations relative to conventional teaching methods. Subsequent research may mitigate these constraints by utilizing longitudinal designs, involving larger and more diverse populations, and adopting experimental or comparative methodologies.

Notwithstanding these constraints, the study offers significant insights into the pedagogical framework and classroom implementation of AI-enhanced simulations in chemistry teaching, establishing a robust basis for subsequent research.

5.2. Acknowledgements

The authors would like to thank the school administrators and chemistry instructors who took part for facilitating the implementation process and providing logistical support during the classroom application. The authors also thank the student participants for their engagement and valuable feedback, which contributed to the practical evaluation of the AtomVerse simulation in an authentic learning environment.

5.3. Funding source

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In accordance with Periódico Tchê Química's ethical guidelines, which prohibit donations from authors with manuscripts under review (even when research funds are available), or in cases of author financial constraints, publication costs were fully absorbed by the journal under our Platinum Open Access policy, with support from Araucária Scientific Association (https://acaria.org/). This policy ensures complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equitable knowledge dissemination.

5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

Raw data are available upon request from the corresponding author (billurwork@gmail.com) due to participant confidentiality and ethical restrictions.

5.6. Author Contributions

Billur Köfter: Conception and design, data collection, data analysis and interpretation, manuscript writing, final approval.

Fatma Alkan: Conception and design, data analysis and interpretation, critical review, final approval.

Ayşem Seda Yücel: Conception and design, critical review, final approval.

5.7. AI and Computational Tools Declaration

Tool: ChatGPT (OpenAI). Purpose: Language refinement, structural editing, and assistance with initial drafting of selected manuscript sections. Extent: Approximately 10% of manuscript preparation. All AI-generated content used during manuscript preparation was reviewed, critically revised, and approved by the authors.

In addition, the AI-based interactive simulation examined in this study (AtomVerse) was developed and implemented on the Replit platform using the GPT-4.0 model as part of the research design. The use of artificial intelligence in the simulation constituted the subject of the study itself.

No artificial intelligence tools were used for data fabrication, statistical analysis, interpretation of results, or scientific decision-making.

5.8. Research Integrity Declaration

The authors certify that this research complies with the standards of research integrity, including no data fabrication, no results falsification, no p-hacking or selective reporting, originality, not previously published, and ethical methods.

An earlier version of the study design was presented as an oral presentation at the ICETOL Conference (August 26–29, 2025); however, the present manuscript includes substantial revisions, expanded implementation, pilot data, and extended analysis, and has not been previously published in any journal.

5.9. Originality & Plagiarism Statement

This manuscript was screened for plagiarism using Turnitin. The overall similarity index was 13%, which is below the acceptable threshold. The authors declare that this manuscript is original work and has not been previously published or submitted elsewhere for consideration. No unattributed text has been copied from other sources, and all references have been properly cited in accordance with APA guidelines. The authors further confirm that this manuscript is not currently under review by any other journal.

5.10. Open Access License

6. STUDIES INVOLVING HUMANS AND ANIMALS

6.1. Ethics Committee Approval

The Hacettepe University Graduate School of Educational Sciences Research Ethics Committee approved this study. Ethical approval was granted under application number E-51944218-050-00004774535 on 17 February 2026, following full committee review.

All procedures involving human participants were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians, and written voluntary participation forms were obtained from all student participants prior to data collection.

6.2. Informed Consent

Written informed consent was obtained from parents or legal guardians of all underage participants, and written voluntary participation forms were obtained from all students prior to data collection. Participants were fully informed about the study's purpose, procedures, and scope.

All data were collected anonymously, and no personally identifiable information was recorded. Participants were informed that their involvement was entirely voluntary, that they could withdraw at any time without any consequences, and that participation would have no impact on their academic standing. All collected data were used solely for research purposes and securely stored to ensure confidentiality and privacy.

7. REFERENCES:

1. Cardoso, P. S. S., Nunes, M. C. S., Silva, G. P. S., Braghittoni, L. S., & Trindade, N. M. (2020). Conceptions of high school students on atomic models, radiation and radioactivity. *Physics Education, 55*(3), 035030.
2. Erümit, A. K., & Sarıalioğlu, R. Ö. (2025). Artificial intelligence in science and chemistry education: A systematic review. *Discover Education, 4*(1), 178.
3. Fabrigas, E. J. A., & Paglinawan, J. L. (2024). Digital simulation exposure and conceptual understanding in chemistry. *International Journal of Research and Innovation in Social Science, 8*(12), 741–747.
4. Göçer, M., Mustafaoğlu, F. M., & Alkan, F. (2026). Use of Web 2.0 Tools in Science Education: Designing Multimedia Learning Environments with the ADDIE Model. In *AI-Powered Educational Games and*

Simulations (pp. 117-158). IGI Global Scientific Publishing.

5. Haryadi, R., & Pujiastuti, H. (2020, April). *PhET simulation software-based learning to improve science process skills.* In *Journal of Physics: Conference Series, 1521*(2), 022017. IOP Publishing.
6. Iyamuremye, A., Niyonzima, F. N., Mukiza, J., Twagilimana, I., Nyirahabimana, P., Nsengimana, T., … & Nsabayezu, E. (2024). Utilization of artificial intelligence and machine learning in chemistry education: a critical review. *Discover Education, 3*(1), 95.
7. Justi, R., & Gilbert, J. (2000). History and philosophy of science through models: some challenges in the case of 'the atom'. *International journal of science education*, *22*(9), 993-1009.
8. Kaya, A. (2023). Addressing Student Misconceptions about Atoms and Examining Instructor Strategies for Overcoming Them. *Journal of Pedagogical Research*, *7*(4), 251-262.
9. Kefalis, C., Skordoulis, C., & Drigas, A. (2025). Digital simulations in STEM education: Insights from recent empirical studies, a systematic review. *Encyclopedia, 5*(1), 10.
10. Köfter, B., Altundağ, C. K., & Yücel, A. S. (2025). A Step Beyond in Industrial Chemistry Education: Digitalization and Sustainability. In *Digital Transformation and Sustainability in Higher Education* (pp. 133-170). IGI Global Scientific Publishing.
11. Maity, S. & Deroy, A. (2024). Generative AI and its impact on personalized intelligent tutoring systems. *arXiv preprint arXiv:2410.10650*.
12. Ministry of National Education (MoNE). (2024). *Chemistry curriculum for upper secondary education (Grades 9–12): Türkiye's Century Education Model*. Directorate General of Board of Education.
13. Malkawi, E. O., Obeidat, S. M., Al-Rawashdeh, N. A., Tit, N., & Obaidat, I. (2018). Misconceptions about atomic models amongst the chemistry students. *International Journal for Innovation Education and Research*, *6*(2), 156-263.
14. Mansoor, N., & Rodríguez, M. A. (2001). Do we have to introduce history and philosophy of science or is it already 'inside' chemistry? *Chemistry Education Research and Practice, 2*(2), 159–164.
15. Moore, E. B., Chamberlain, J. M., Parson, R., & Perkins, K. K**.** (2014, July 2). *PhET Interactive Simulations: Transformative Tools for Teaching Chemistry. Journal of Chemical Education, 91*(8), 1191–1197.
16. Nadelson, L. S., Heddy, B. C., Jones, S., Taasoobshirazi, G., & Johnson, M. (2018). Conceptual change in science teaching and learning: Introducing the dynamic model of conceptual change. *International Journal of Educational Psychology, 7*(2), 151–195.
17. Niaz, M., Aguilera, D., Maza, A., & Liendo, G. (2002). Arguments, contradictions, resistances, and conceptual change in students' understanding of atomic structure. *Science education*, *86*(4), 505-525.
18. Richey, R. C., & Klein, J. D. (2014). *Design and development research: Methods, strategies, and issues*. Routledge.
19. Schank, R. C., Fano, A., Bell, B., & Jona, M. (1994). The design of goal-based scenarios. *The Journal of the Learning Sciences, 3*(4), 305–345.
20. Valeeva, R., Biktagirova, G., Lesev, V., Mikhailenko, O., Skudareva, G., & Valentovinis, A. (2023). *Exploring the impact of modeling in science education: A systematic review*. Eurasia Journal of Mathematics, Science and Technology Education, 19(6), em2284.
21. Vygotsky, L. S., & Cole, M. (1978). *Mind in society: Development of higher psychological processes*. Harvard University Press.
22. Zarkadis, N., Stamovlasis, D., & Papageorgiou, G. (2020). Student ideas and misconceptions for the atom: A Latent Class Analysis with covariates. *International Journal of Physics and Chemistry Education*, *12*(3), 41-47.
23. Wang, S., Wang, F., Zhu, Z., Wang, J., Tran, T., & Du, Z. (2024). Artificial intelligence in education: A systematic literature review. *Expert Systems with Applications, 252*, 124167.
24. Weisi, H., & Zamani, G. (2015). A comparison of the cognitive taxonomy of

educational objectives to the evolution of the atomic theory. *Enjoy Teaching Journal, 3*(3), 1–8.

25. Wieman, C. E., Adams, W. K., & Perkins, K. K. (2008, October 31). PhET: Simulations that enhance learning. *Science, 322*(5902), 682–68

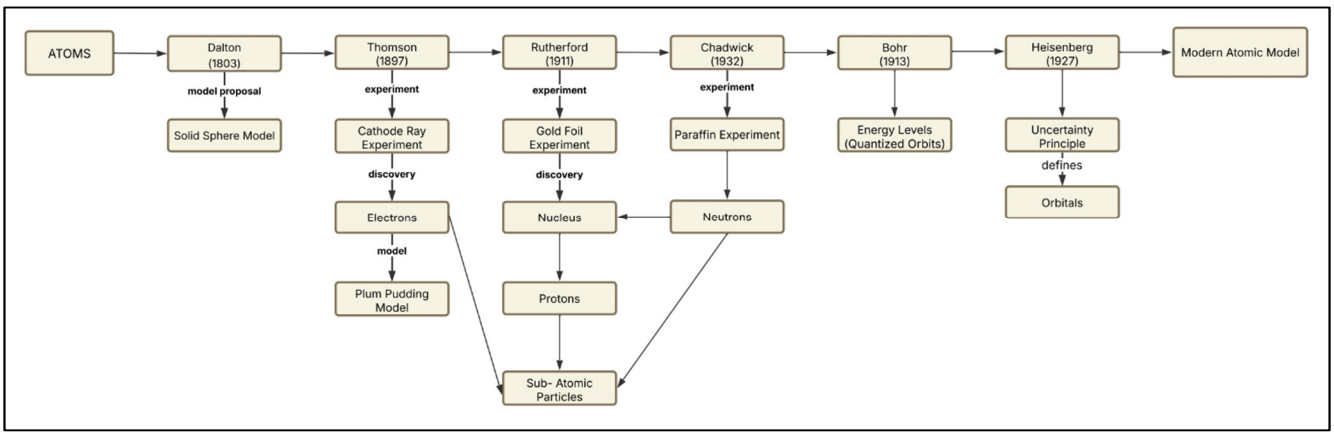

Figure 1: *Concept map representing the historical development of atomic models, showing the contributions of six key scientists (Dalton, Thomson, Rutherford, Chadwick, Bohr, and Heisenberg) and their experimental and theoretical findings.*

Figure 2: *The opening screen introduces students to the simulation and provides access to scientists' dialogues. It serves as the main navigation center where users can start their learning session and enter the assessment module.*

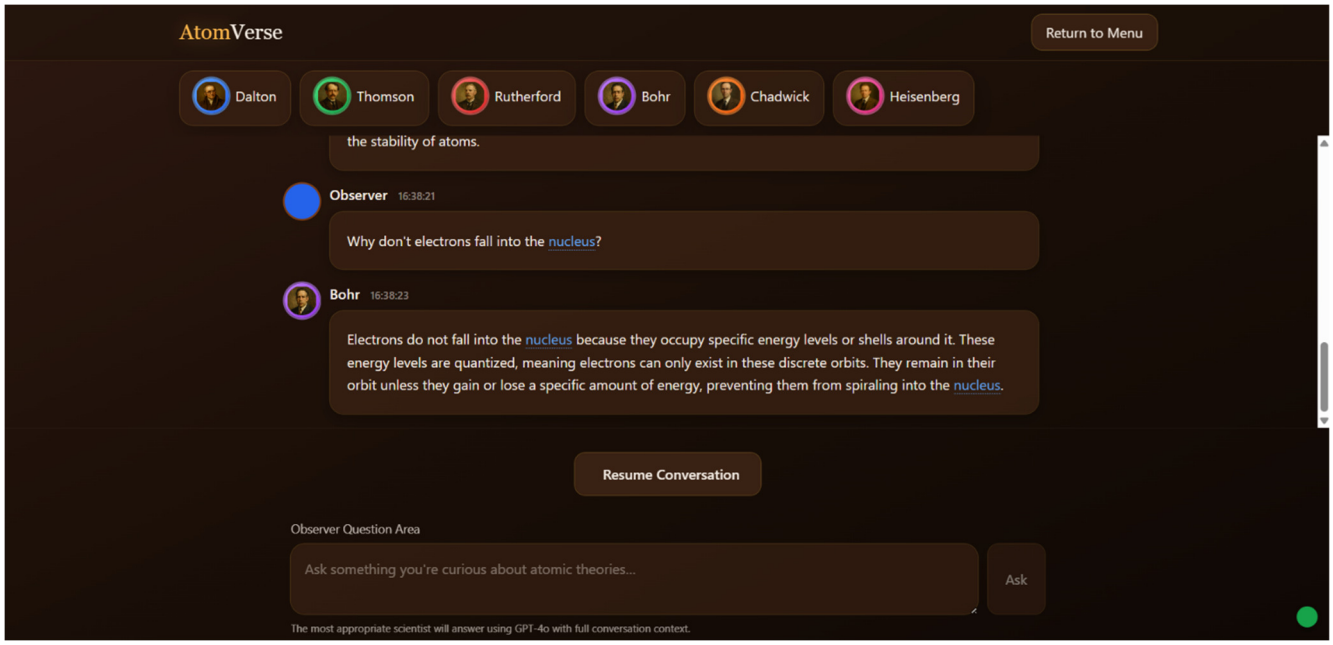

Figure 3: *Interactive dialogue screen in the AtomVerse simulation. The student asks a scientist a direct question and receives a response generated by artificial intelligence. This environment enables real-time interaction.*

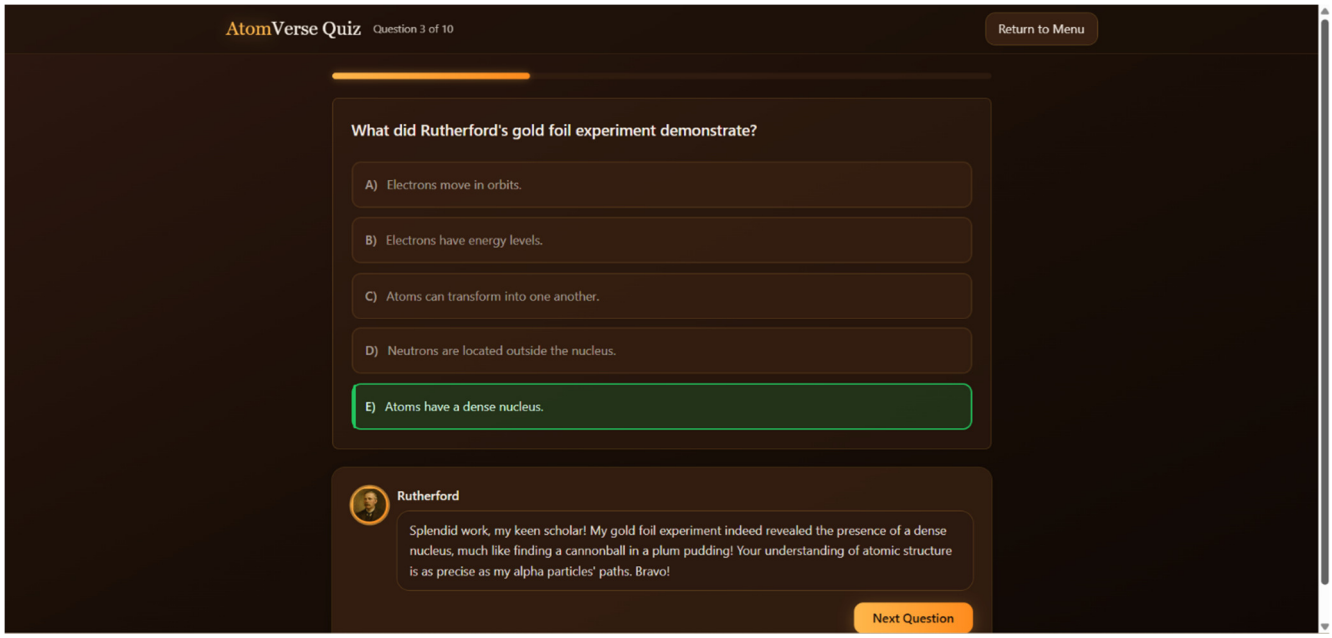

Figure 4: *An example screen from the AtomVerse test module. The test module enables students to answer conceptual questions and receive automatic feedback, helping assess their understanding after completing the simulation.*

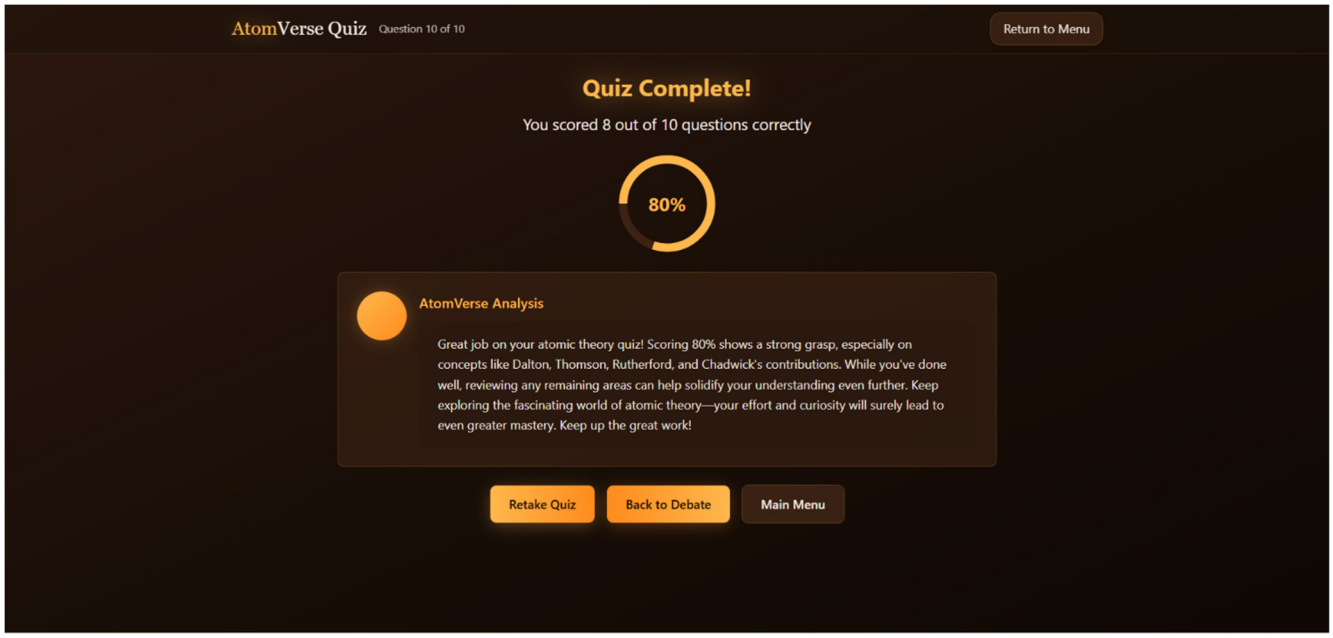

Figure 5: *The evaluation screen summarizes students' overall performance in the test module simulation with artificial intelligence support; it displays students' scores, feedback, and progress in conceptual understanding.*

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

AVALIAÇÃO DOS NÍVEIS DE ELETRÓLITO SÉRICO EM PACIENTES COM DIABETES MELLITUS

ASSESSMENT OF SERUM ELECTROLYTE LEVELS IN DIABETES MELLITUS PATIENTS

تقييم مستويات الشوارد في مصل الدم لدى مرضى داء السكري

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RESUMO

Introdução: Neste estudo, foram estimados novos biomarcadores para concentrações de alguns eletrólitos e sua relação com o diabetes. **Objetivo**: O estudo visa avaliar as concentrações críticas de alguns eletrólitos importantes presentes no corpo e sua relação com pacientes com diabetes tipo 2, além de estudar as razões para a diminuição e aumento de algumas concentrações. **Métodos:** Uma amostra foi retirada de pacientes com diabetes tipo 2 e as concentrações de alguns íons importantes foram medidas. Alguns desses íons foram estudados usando um dispositivo de eletrólitos SEMI do modelo American Origin, que foi então usado para testar as concentrações de eletrólitos. **Resultados:** Um total de 100 participantes foi incluído neste estudo, composto por 50 pacientes com diabetes mellitus tipo 2 (T2D) e 50 indivíduos saudáveis pareados por idade (ND). A idade dos participantes variou entre 15 e 65 anos. As avaliações bioquímicas incluíram a mensuração dos níveis de cálcio, cloreto, sódio e potássio em ambos os grupos. Entre os participantes, 35 (70%) eram do sexo masculino e 15 (30%) do sexo feminino. Não foi observada diferença estatisticamente significativa ($p > 0,05$) entre os grupos T2D e ND quanto à distribuição por sexo. **Discussão:** Os desequilíbrios eletrolíticos podem resultar do uso de medicamentos como diuréticos, medicamentos antidiabéticos e insulina exógena, bem como hiperglicemia, insuficiência renal e cetoacidose, outras condições que alteram a concentração de eletrólitos no corpo. Em contraste com os pacientes ND, os níveis séricos de Na^{+} e Cl^{-} aumentaram significativamente ($p < 0,05$) nesta investigação, embora os aumentos nos níveis de Ca^{+2} e K^{+} não tenham sido estatisticamente significativos ($p>0,05$). Esse achado está de acordo com pesquisas anteriores que mostraram níveis elevados de Na^{+} e Cl^{-} em pacientes com diabetes devido ao aumento da perda de água por diurese osmótica. No entanto, não houve alteração perceptível no Ca^{+2} entre os indivíduos DM2 e ND (valor de $P > 0,05$). **Conclusões:** A homeostase da glicose pode ser comprometida devido a alterações nos níveis de sódio, potássio, cálcio e cloreto.

Palavras-chave:*Idade, Diabetes mellitus, Concentração de eletrólitos, HbA1C*

ABSTRACT

Background: In this study, new biomarkers for concentrations of some electrolytes and their relationship to diabetes were estimated**. Aim:** The study aims to assess the critical concentrations of some important electrolytes present in the body and their relationship to patients with type 2 diabetes and to study the reasons for the decrease and increase in some concentrations**. Methods:** A sample was drawn from patients with type 2 diabetes, and the concentrations of some important ions were measured. Some of these ions were studied using an American Origin Model SEMI electrolyte device that was then used to test the electrolyte concentrations. **Results**: A total of 100 participants were included in this study, comprising 50 patients with type 2 diabetes (T2D) and 50 age-matched healthy controls (ND). The participants ranged in age from 15 to 65 years. Biochemical assessments included measurements of calcium, chloride, sodium, and potassium levels in both groups. Among the participants, 35 (70%) were male and 15 (30%) were female. No statistically significant difference ($p > 0.05$) was observed between T2D and ND groups with respect to sex distribution. **Discussion**:

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Electrolyte imbalances can result from using of drugs such as diuretics, antidiabetic medications, and exogenous insulin, as well as hyperglycemia, renal failure, and ketoacidosis, other conditions that alter the body's concentration of electrolytes. In contrast to ND patients and T2D patients, serum Na^+ and Cl^- levels increased significantly ($p < 0.05$) in this investigation, although the increases in Ca^{+2} and K^+ levels were not statistically significant ($p > 0.05$). This finding is in line with previous research that showed elevated Na+ and Cl^- levels in diabetes patients due to increased water loss via osmotic diuresis. However, there was no discernible change in Ca^{+2} between the T2D and ND individuals (P value > 0.05). **Conclusions:** Glucose homeostasis may be disrupted due to alterations in sodium, potassium, calcium, and chloride levels.

Keywords: *Age, Diabetes mellitus, Electrolytes concentration, HbA1C.*

الملخص

الخلفية: في هذه الدراسة، جرى تقدير مؤشرات حيوية جديدة لتراكيز بعض الشوارد وعلاقتها بداء السكري. **الهدف:** تهدف هذه الدراسة إلى تقييم التراكيز الحرجة لبعض الشوارد المهمة في الجسم وعلاقتها بمرضى السكري من النوع الثاني، فضلًا عن دراسة أسباب انخفاض وارتفاع بعض هذه التراكيز. **طرائق العمل:** تم سحب عينات من مرضى السكري من النوع الثاني، وقياس تراكيز بعض الأيونات المهمة. وقد دُرست هذه الأيونات باستخدام جهاز الشوارد من نوع SEMI ذو منشأ أمريكي، والذي استُخدم لقياس تراكيز الشوارد. **النتائج:** شملت هذه الدراسة 100 مشاركًا، منهم 50 مريضًا بداء السكري من النوع الثاني (T2D) و50 فردًا سليمًا متوافقين في العمر (ND). وتراوحت أعمار المشاركين بين 15 و65 عامًا. وقد شملت التقييمات الكيميائية الحيوية قياس مستويات الكالسيوم والكلوريد والصوديوم والبوتاسيوم في كلا المجموعتين. ومن بين المشاركين، كان 35 (70%) من الذكور و15 (30%) من الإناث، ولم تُسجَّل فروق ذات دلالة إحصائية ($p > 0.05$) بين مجموعتي مرضى السكري وغير المصابين من حيث التوزيع الجنسي. **المناقشة:** يمكن أن تنجم اختلالات الشوارد عن استخدام أدوية مثل المدرّات، والأدوية المضادة للسكري، والإنسولين الخارجي، إضافةً إلى فرط سكر الدم، والفشل الكلوي، والحماض الكيتوني، وغيرها من الحالات التي تؤثر في تراكيز الشوارد في الجسم. وقد أظهرت هذه الدراسة ارتفاعًا معنويًا ($p < 0.05$) في مستويات الصوديوم (Na^+) والكلوريد (Cl^-) في المصل لدى مرضى السكري من النوع الثاني مقارنةً بغير المصابين، في حين لم تكن الزيادات في مستويات الكالسيوم (Ca^{+2}) والبوتاسيوم (K^+) ذات دلالة إحصائية ($p > 0.05$). وتتوافق هذه النتائج مع دراسات سابقة أظهرت ارتفاع مستويات الصوديوم والكلوريد لدى مرضى السكري نتيجة زيادة فقدان الماء عبر الإدرار الأسموزي. كما لم يُلاحظ تغير معنوي في مستوى الكالسيوم (Ca^{+2}) بين المجموعتين ($p > 0.05$). **الاستنتاجات:** قد يختلّ الاتزان الداخلي للغلوكوز نتيجة التغيّرات في مستويات الصوديوم والبوتاسيوم والكالسيوم والكلوريد.

الكلمات المفتاحية: العمر، داء السكري، تراكيز الشوارد، *HbA1C*

1. INTRODUCTION:

The body uses electrolytes, such as K^+, Na^+, Cl^-, and Ca^{2+}, to promote various metabolic processes that ensure normal homeostasis and cellular activity and improve the creation of electrical gradients and enzyme activity (Chanchlani *et al*., 2017). However, deviations from normal electrolyte levels or imbalances can cause scientific illnesses or anomalies that are regularly related to lower mortality and morbidity rates (Coregliano-Ring *et al*., 2022).

Electrolyte imbalances are a common observation in clinical patients. They can be caused by various factors, including gastrointestinal absorption capacity, pharmaceutical medications, abnormalities in base acidity, acute medical conditions, or diseases that may function independently or in combination (Timerga *et al*.,2020). One of the conditions that commonly causes electrolyte distortion is diabetes (Kataoka *et al*.,2020). High blood glucose levels in diabetics cause an increase in plasma osmolality, which produces an osmotic driving force that causes water to flow from intracellular to extracellular areas (Zhang *et al*., 2022). Diabetes mellitus can lead to disturbances in electrolyte balance, which may contribute to complications (Muthmainnah et al., 2021). Monitoring serum electrolytes is important in the management of patients with diabetes (Yumashev *et al*., 2019; Al-Kaaby & Al-Ali, 2023; QASIM & FALIH., 2020).

There are two main ways this water movement and osmotic drift affect the body's concentration of electrolytes (Eledrisi *et al*., 2020), (Elliott *et al*., 2024). If the electrolyte concentration is extracellular, it may dilute or increase depending on whether intracellular electrolytes are carried to the extracellular space by water movement, particularly in the event of insulin insufficiency (Egboh *et al*., 2022). An electrolyte imbalance or disease is the result of this osmotic drift. Diabetes is linked to both hyper and hypo-electrolyte levels (Ye *et al*.,2016). Because so little research has assessed the degree of chloride modification across different groups, the dysregulation of chloride in diabetes is still unknown (Lee *et al*.,2020). Diabetic nephropathy, one of the consequences of diabetes marked by reduced renal function or failure, can cause an electrolyte imbalance (Khan *et al*., 2019). Diabetes is a complex illness that has correlations with age, sex, blood pressure,

and other variables (Shridhar *et al*., 2020; Wang *et al*., 2013).

Therefore, the study aims to evaluate the serum electrolyte level of patients with diabetes to assess the correlation of this electrolyte with diabetes risk factors.

1.1. Aims

The study aims to assess the critical concentrations of some important electrolytes present in the body and their relationship to patients with type 2 diabetes and to study the reasons for the decrease and increase in some concentrations.

1.2. Study Hypothesis

A hypothesis is an initial idea or a prospect that entails proving or disproving a causal relationship in social life between primary and secondary variables through experimental testing. It is written in a distinctive style that expresses the researcher's researcher's views regarding a particular issue. Hypotheses are a collection of opinions and ideas gained from reality and arranged rationally. The researcher thus picked a single hypothesis, which is as follows: (Chronic diabetic disease has a clear effect on the concentration of electrolytes in blood and hence creates numerous health problems). This hypothesis was chosen based on its implications to the theoretical side and factual facts in Iraq.

2. MATERIALS AND METHODS:

Provide sufficient details to permit repetition of the experimental work. A technical description of the methods should be given when they are new.

2.1. Materials

Blood samples were collected from patients with type 2 diabetes and healthy people, and the samples consisted of serum Cl^-, Na^+, K^+, and Ca^{+2}.

2.1.1. Study Samples

The population sample, which consists of some people we believe to share the same traits as those in the study group, is intended to be a specific, quantitative, and qualitative component. Consequently, a sample of 50 individuals with diabetes and 50 healthy individuals (control group) was purposefully selected.

2.2. Methods

Blood samples (5 mL) were collected from 50 diabetic patients and 50 healthy individuals using serum separator tubes. The samples were allowed to clot at room temperature for 20 minutes and then centrifuged at 3500 rpm (or specify the relative centrifugal force) for 15 minutes to separate the serum. Using a micropipette, the serum was extracted (1 ml) and transferred to microcentrifuge tubes. The serum samples were stored at -20°C until electrolyte concentrations were determined Within 24 hours. An American Origin Model SEMI electrolyte analyzer (fully automated random access clinical chemistry analyzer with photometric throughput of 400 tests / hour) was used to measure the concentrations of sodium, potassium, chloride, and calcium in the serum samples. The device was calibrated according to the manufacturer's guidelines, and quality control measures were performed using standard solutions before sample analysis.

2.1.1 Data on the distribution of sex

This section presents data on the sex distribution of the study participants, which refers to the biological classification of individuals as either male or female. An individual's sex can directly influence the results of a study due to biological differences between males and females. According to the statistical data, 40 out of the 50 diabetes patients in this study are men, while the remaining 10 patients are women, as indicated in Table 1 of the study's results.

2.1.2. The period of diabetes

The duration of diabetes is one of the factors affecting the results and data of this study, as concentrations of electrolytes in blood serum differ depending on the duration of diabetes. In the research, the patients were divided into three categories based on the duration of their diabetes: the first group includes patients with a disease duration of 1-5 years, the second group includes those with a duration of 6-10 years, and the third group includes those with a duration of 11-15 years, as shown in Table 2.

2.1.3. *Statistics*

Statistical analysis of the data was conducted using SPSS version 19. The analysis included descriptive statistics of the study population, types of samples, and methods of selecting them. Data were presented in tables,

and appropriate statistical tests, such as anova test, were applied to the primary and secondary data collected.

3. RESULTS AND DISCUSSION:

3.1. Results

There were 100 participants in this study, 50 of them had diabetes, and the other 50 were age-matched healthy controls. Table 3 displays the results of biochemical assays, such as calcium, chloride, sodium, and potassium levels, for both the diabetes and control groups. In the patients, HbA1c was considerably (P value < 0.05) more than that of non-diabetic healthy controls. Sodium and chloride levels in diabetic patients were higher than in controls, and these differences were significant (P value < 0.05). The calcium and potassium levels were found to be marginally higher than those of the controls.

A total of 100 participants participated in the trial; 50 (or 50%) of them had T2D, while the remaining 50 (or 50%) were ND patients, ages 15 to 65. There were 35 (70%) male and 15 (30%) female patients among them, and there was no discernible ($p>0.05$) sex difference between the T2D and ND patients. Table 4 indicates that the T2D patients had significantly higher age and FBS ($p<0.05$) than the ND patients, although the differences in SBP and DBP were not statistically significant. The periods were divided into three-time categories, where the first category included those affected from 1 to 5 years, the category from 6 to 10 years, and the third category from 11 to 15 years. Analyzes using Anova statistical analysis showed an evident variation in electrolyte concentrations, according to Table 5, Table 6, Table 7, and Table 8.

3.2. Discussion

Electrolyte imbalances can result from using of drugs such as diuretics, antidiabetic medications, and exogenous insulin, as well as hyperglycemia, renal failure, and ketoacidosis, other conditions that alter the body's concentration of electrolytes. In contrast to ND patients T2D patients, serum Na^+ and Cl^- levels increased significantly ($p<0.05$) in this investigation, although the increases in Ca^{+2} and K^+ levels were not statistically significant ($p>0.05$).

This finding is in line with previous research that showed elevated Na^+ and Cl^- levels in diabetes patients due to increased water loss via osmotic diuresis. However, there was no discernible change in Ca^{+2} between the T2D and ND individuals ($P >0.05$). This result was also seen in another investigation where Cl- significantly ($P < 0.05$) linked clearly with SBP and DBP. Nevertheless, no correlation found between (SBP and DBP) Ca^{+2} and K^+. Elevations of K^+ can result from elevated blood sugar since FBS and K^+ have a positive correlation. This, however, is not the usual situation, as hypokalemia is typically caused by hyperglycemia.

Consistent with our findings, Muthmainnah *et al.* (2021) also reported that patients with a family history of diabetes had a higher risk of developing gestational diabetes. The use of mesodiencephalic modulation therapy was shown by Yumashev *et al.* (2019) to help regulate blood glucose and improve outcomes in diabetic patients undergoing dental procedures, supporting the potential for adjunctive non-pharmacological interventions.

4. CONCLUSIONS:

In the current study, we found significantly high levels of sodium, potassium, calcium, and chloride in patients with type II diabetes when compared with healthy controls. All the comparisons had a p-value less than 0.05. Electrolyte abnormalities were associated with disrupted glucose homeostasis. Alterations in electrolyte balance could, therefore, play a role in the pathophysiology of type II diabetes. The results highlight the need for checking the levels of electrolytes in patients with type II diabetes, since these abnormalities may sometimes affect the prognosis or management of the disease. Further investigations should therefore seek to determine the nature of the underlying mechanisms that relate electrolyte disturbances to glucose homeostasis in T2D and possible clinical utility arising from monitoring of electrolytes. Interventions targeted at modifying electrolyte balance may be a new approach to therapy in improving outcomes in patients with type II diabetes, but further research is needed to explore this.

5. DECLARATIONS

5.1. Study Limitations

limitations include the small sample size, lack of robust demographic and clinical data, limited generalizability as a single-center study, and absence of longitudinal follow-up and outcome assessments.

5.2. Acknowledgments

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5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author.

5.6. Author Contributions

Ayad Kadhim: Conception and design, manuscript writing, final approval. Wisam Okash: Data collection, analysis, and interpretation, final approval.

5.7. AI and Computational Tools Declaration

"The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation."

5.8. Research Integrity Declaration

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5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

'Retrospective study using anonymized secondary data'.

6.2. Informed Consent

Not applicable.

7. REFERENCES:

1. Chanchlani, R., Joseph Kim, S., Kim, E. D., Banh, T., Borges, K., Vasilevska-Ristovska, J., ... & Parekh, R. S. (2017). Incidence of hyperglycemia and diabetes and association with electrolyte abnormalities in pediatric solid organ transplant recipients. *Nephrology Dialysis Transplantation*, *32*(9), 1579-1586. DOI: 10.1093/ndt/gfx205

2. Coregliano-Ring, L., Goia-Nishide, K., & Rangel, É. B. (2022). Hypokalemia in diabetes mellitus setting. Medicina, 58(3), 431. DOI: 10.3390/medicina58030431

3. DEVI, Y. P., ABDILLAH, R., & MUTHMAINNAH, M. (2021, March 20). DETERMINANTS OF GESTATIONAL DIABETES MELLITUS. Periódico Tchê Química. Dr. D. Scientific Consulting. DOI: 10.52571/PTQ-v18-n73-pgi.48-2021

4. Timerga, A., Kelta, E., Kenenisa, C., Zawdie, B., Habte, A., & Haile, K. (2020). Serum electrolytes disorder and its associated factors among adults admitted with metabolic syndrome in Jimma Medical Center, South West Ethiopia: Facility based crossectional study. PloS one, 15(11), e0241486. DOI: 10.1371/journal.pone.0241486

5. Kataoka, H., & Yoshida, Y. (2020). Enhancement of the serum chloride concentration by administration of sodium–glucose cotransporter-2 inhibitor and its mechanisms and clinical significance in type 2 diabetic patients: a pilot study. Diabetology & Metabolic Syndrome, 12, 1-7. DOI: 10.1186/s13098-020-0515-x

6. Zhang, J., Huan, Y., Leibensperger, M., Seo, B., & Song, Y. (2022). Comparative effects of sodium-glucose cotransporter 2 inhibitors on serum electrolyte levels in patients with type 2 diabetes: a pairwise and network meta-analysis of randomized controlled trials. Kidney360, 3(3), 477-487. DOI: 10.34067/KID.0006672021

7. YUMASHEV, A., MATVEEVA, E., TAMBOVTSEVA, N., LI, J., & YANG, B. (2019, March 20). THERAPEUTIC AND PROPHYLACTIC APPLICATION OF MESODIENCEPHALIC MODULATION DURING DENTAL IMPLANTATION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. Periódico Tchê Química. Dr. D. Scientific Consulting. DOI:10.52571/PTQ.v16.n33.2019.97_Periodico33_pgs_82_93.pdf

8. Eledrisi, M. S., & Elzouki, A. N. (2020). Management of diabetic ketoacidosis in adults: a narrative review. Saudi journal of medicine & medical sciences, 8(3), 165-173. DOI: 10.4103/sjmms.sjmms_478_19

9. Egboh, V. O., Ohwin, P. E., Daubry, T. M. E., Ofulue, O. O., Nwogueze, B. C., Ojugbeli, E. T., ... & Nwangwa, E. K. (2022). Comparative Analysis of Fasting Blood Glucose and Salivary Electrolytes Concentrations among Individuals with Type II Diabetes: A Randomized Controlled Hospital Based Study. *Toxicology Reports*, *9*, 1268-1272. DOI: 10.1016/j.toxrep.2022.05.022

10. Lee, I. H., & Ahn, D. J. (2020). Dapagliflozin-associated euglycemic diabetic ketoacidosis in a patient with type 2 diabetes mellitus: a case report. Medicine, 99(21), e20228. DOI: 10.1097/MD.0000000000020228

11. Khan, R. N., Saba, F., Kausar, S. F., & Siddiqui, M. H. (2019). Pattern of electrolyte imbalance in Type 2 diabetes patients: Experience from a tertiary care hospital. Pakistan Journal of Medical Sciences, 35(3), 797. DOI: 10.12669/pjms.35.3.844

12. Shridhar, R., Sushith, S., Balakrishna, P. M., D'Sa, J., Pragathi, G., PK, K. K., ... & Sukhlal, K. B. (2020). Serum electrolytes levels in patients with type 2 diabetes mellitus: a cross-sectional study. Сахарный диабет, 23(3), 223-228. DOI: 10.18632/oncotarget.23208

13. Wang, S., Hou, X., Liu, Y., Lu, H., Wei, L., Bao, Y., & Jia, W. (2013). Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus. Cardiovascular diabetology, 12, 1-10.
DOI: 10.1186/1475-2840-12-146

14. Ye, S., Ruan, P., Yong, J., Shen, H., Liao, Z., & Dong, X. (2016). The impact of the HbA1c level of type 2 diabetics on the structure of haemoglobin. Scientific Reports, 6(1), 33352.
DOI: 10.1038/srep33352

15. Elliott, A., Walters, R. K., Pirinen, M., Kurki, M., Junna, N., Goldstein, J. I., ... & Widén, E. (2024). Distinct and shared genetic architectures of gestational diabetes mellitus and type 2 diabetes. Nature Genetics, 1-6.
DOI: 10.1038/s41588-023-01607-4

16. Al-Kaaby, H. H., & AL-Ali, Z. A. R. (2023, June 9). TYPE 2 DIABETES MELLITUS EFFECTS ON SEMEN PARAMETERS AND SEMINAL PLASMA. SOUTHERN JOURNAL OF SCIENCES. Southern Brazilian Journal of Chemistry. Retrieved from DOI:10.48141/SJS.v31.n35.2023.03_KAABY_pgs_16_25.pdf

17. QASIM, M. J., & FALIH, I. Q. (2020, December 20). REVIEW ABOUT DIABETES MELLITUS AND URINARY TRACT INFECTIONS. SOUTHERN BRAZILIAN JOURNAL OF CHEMISTRY. Southern Brazilian Journal of Chemistry. Retrieved from DOI:10.48141/SBJCHEM.v28.n29.2020.02_QASIM_pgs_07_16.pdf

Table 1. *Gender distribution of samples*

Gender	diabetes patients		control group	
	Number	Percentage (%)	Number	Percentage (%)
Males	40	80%	35	70%
Females	10	20%	15	30%
Total	50	100%	50	100%

Table 2. *The Period of Diabetes Distribution of Samples*

Period of diabetes	Number	Percentage (%)
(one - five) years	fifteen	40 %
(six - ten) years	twenty	30 %
(eleven - fifteen) years	fifteen	30 %
total	fifty	100 %

Table 3. *Biochemical Measurements in Type II Diabetes*

Parameters	Diabetes (50) mean ± SD	Controls (50) mean ± SD	P* Value
**HbA1C(%)	10.8±1.41	4.6±0.5	< 0.05
Na^{+} mmol/L	141.44±6.78	134.98±13.68	< 0.05
K^{+} mmol/L	3.82±0.34	4.16±0.38	< 0.05
Ca^{+2} mmol/L	1.05±0.05	1.07±0.08	< 0.05
Cl^{-} mmol/L	102.18±4.55	96.96±11.01	< 0.05

P * value < 0.05 then it is considered to be statistically significant

**HbA_1c: Glycosylated Hemoglobin

Table 4. *Mean age, duration, SBP, DBP and FBS of diabetes mellitus in the study group.*

Variables	minimum	maximum	T2D Mean ±SD	ND Mean ±SD	P-value
Age(year)	15	65	40.0±14.86	39.54±15.23	0.050
Duration(year)	1	15	8.0±4.47	----------	p<0.05
SPB*(mmHg)	105	218	133.32±2.19	120.29±0.24	0.768
DPB**(mmHg)	63	156	80.21±2.32	80.21±0.16	0.230
FBS***(mg/dL)	16.0	320.0	176.21±13.72	66.57±2.32	p<0.05

***** (SBP: Systolic Blood Pressure); ****(**DBP: Diastolic Blood Pressure); ******* (FBS: Fasting Blood Sugar)

Table 5. *Potassium ion concentration and time period of diabetes*

Element	(I) Time period of diabetes	(J) Time period of diabetes	Mean Difference (I-J)	Std. Error	Sig.
K^+	(1 - 5) years	(6- 10) years	0.16	0.11	0.36
		(11 - 15) years	0.34	0.11	0.02
	(6- 10) years	(1 - 5) years	0.16	0.11	0.36
		(11 - 15) years	0.18	0.11	0.26
	(11 - 15) years	(1 - 5) years	0.34	0.11	0.02
		(6- 10) years	0.18	0.11	0.26

Table 6. *Sodium ion concentration and time period of diabetes*

Element	(I) Time period of diabetes	(J) Time period of diabetes	Mean Difference (I-J)	Std. Error	Sig.
Na^+	(1 - 5) years	(6- 10) years	0.83	0.38	0.10
		(11 - 15) years	1.75	0.40	0.00
	(6- 10) years	(1 - 5) years	0.83	0.38	0.10
		(11 - 15) years	0.91	0.38	0.06
	(11 - 15) years	(1 - 5) years	1.75	0.40	.000
		(6- 10) years	0.91	0.38	0.00

Table 7. *Calcium ion concentration and time period of diabetes*

Element	(I) Time period of diabetes	(J) Time period of diabetes	Mean Difference (I-J)	Std. Error	Sig.
Ca^{2+}	(1 - 5) years	(6- 10) years	0.12	0.01	.000
		(11 - 15) years	0.22	0.01	.000
	(6- 10) years	(1 - 5) years	0.12	0.01	.000
		(11 - 15) years	0.1	0.01	.000
	(11 - 15) years	(1 - 5) years	0.22	0.01	.000
		(6- 10) years	0.10	0.01	.000

Table 8. *Chloride ion concentration and time period of diabetes*

Element	(I) Time period of diabetes	(J) Time period of diabetes	Mean Difference (I-J)	Std. Error	Sig.
Cl^{-}	(1 - 5) years	(6- 10) years	0.69	0.71	0.62
		(11 - 15) years	0.08	0.76	0.99
	(6- 10) years	(1 - 5) years	0.69	0.71	0.62
		(11 - 15) years	0.77	0.71	0.55
	(11 - 15) years	(1 - 5) years	0.08	0.76	0.99
		(6- 10) years	0.77	0.71	0.55

PERIÓDICO TCHÊ QUÍMICA
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CARACTERÍSTICAS COPROLÓGICAS DA INFECÇÃO POR *BLASTOCYSTIS* SPP.: UM ESTUDO COMPARATIVO DA FEDERAÇÃO RUSSA

COPROLOGICAL CHARACTERISTICS OF *BLASTOCYSTIS* SPP. INFECTION: A COMPARATIVE STUDY FROM THE RUSSIAN FEDERATION

КОПРОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ ИНФЕКЦИИ *BLASTOCYSTIS* SPP.: СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ИЗ РОССИЙСКОЙ ФЕДЕРАЦИИ

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RESUMO

Introdução: As parasitoses intestinais, especialmente a blastocistose causada por Blastocystis spp., representam um problema de saúde global, afetando até 80% da população em países em desenvolvimento. A compreensão dos distúrbios digestivos funcionais associados a essa infecção é crucial para o diagnóstico e tratamento. **Objetivo:** Investigar as alterações estruturais e químicas no trato gastrointestinal durante a infecção por Blastocystis spp. por meio do exame coprológico completo e avaliar o valor diagnóstico de seus indicadores. **Métodos:** Foi realizado um estudo transversal com 503 indivíduos com doenças gastrointestinais e infecção confirmada por Blastocystis spp., comparados a um grupo controle de 150 indivíduos saudáveis pareados por sexo e idade. Amostras fecais foram analisadas sem padronização dietética prévia, utilizando quatro preparações: nativa, com solução de Lugol, com Sudan e com glicerina. A análise incluiu a avaliação de detritos, fibras musculares e vegetais, amido e a identificação de protozoários. Resultados: Indivíduos infectados apresentaram distúrbios digestivos significativos em comparação aos controles. Observou-se redução do conteúdo de detritos em mais de 50% dos infectados, indicando digestão prejudicada. Mais de 80% exibiram fibras musculares cilíndricas mal digeridas. A fibra vegetal digestível estava elevada, variando com a consistência das fezes ($p < 0,05$). A ausência completa de digestão do amido foi observada em 100% dos infectados, com 56,85% apresentando quantidades significativas (++) e 43,15% quantidades muito grandes (+++), contrastando com 98% do grupo controle sem amido detectável ($p < 0,001$). **Discussão:** Os achados indicam que a infecção por Blastocystis spp. causa alterações profundas nos processos digestivos, caracterizadas por fermentação e digestão incompleta de carboidratos e proteínas. O exame coprológico mostrou-se uma ferramenta simples e de alto valor diagnóstico para detectar esses distúrbios funcionais. **Conclusões:** Os indicadores coprológicos são instrumentos laboratoriais eficazes para diagnosticar doenças parasitárias como a blastocistose e podem orientar estratégias terapêuticas na prática clínica.

Palavras-chave: *Blastocystis* spp.; blastocistose; coprograma; doenças gastrointestinais; protozoários.

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ABSTRACT

Background: Intestinal parasitic diseases, particularly blastocystosis caused by *Blastocystis* spp., represent a global health concern, affecting up to 80% of populations in developing countries. Understanding the functional digestive disturbances associated with this infection is crucial for diagnosis and treatment. **Aim**: This study aimed to investigate the structural and chemical changes in the gastrointestinal tract during *Blastocystis* spp. infection using comprehensive coprogram characteristics and to evaluate the diagnostic value of its indicators. **Methods**: A cross-sectional study was conducted on 503 individuals with gastrointestinal diseases and confirmed *Blastocystis* spp. infection, compared with 150 healthy controls matched by sex and age. Fecal samples were analyzed without prior dietary standardization, using four preparations: native, Lugol's solution, Sudan solution, and glycerin. Analysis included evaluation of detritus, muscle and plant fibers, starch, and protozoa identification. **Results**: Infected individuals showed significant digestive disturbances compared to controls. Reduced detritus content was observed in over 50% of infected individuals, indicating impaired digestion. Over 80% exhibited poorly digested cylindrical muscle fibers. Digestible plant fiber was significantly elevated, varying with stool consistency ($p < 0.05$). Complete absence of starch digestion was observed in 100% of infected individuals, with 56.85% showing significant amounts (++) and 43.15% very large amounts (+++), contrasting with 98% of controls with no detectable starch ($p < 0.001$). **Discussion**: The findings indicate that *Blastocystis* spp. infection causes profound alterations in digestive processes, characterized by fermentation and incomplete digestion of carbohydrates and proteins. Coprological examination proved to be a simple, highly valuable diagnostic tool for detecting these functional disorders. **Conclusions**: Coprogram indicators serve as effective laboratory instruments for diagnosing parasitic diseases like blastocystosis and can guide therapeutic strategies in clinical practice.

Keywords: *Blastocystis* spp.; blastocystosis; coprogram; gastrointestinal diseases; protozoa.

АННОТАЦИЯ

Актуальность: Кишечные паразитарные заболевания, особенно бластоцистоз, вызываемый *Blastocystis* spp., представляют глобальную проблему здравоохранения, поражая до 80% населения в развивающихся странах. Понимание функциональных нарушений пищеварения, связанных с этой инфекцией, имеет решающее значение для диагностики и лечения. **Цель**: Изучить структурные и химические изменения в желудочно-кишечном тракте при инвазии *Blastocystis* spp. с помощью развернутого копрологического исследования и оценить диагностическую ценность его показателей. **Методы**: Проведено поперечное исследование с участием 503 человек с заболеваниями желудочно-кишечного тракта и подтвержденной инфекцией *Blastocystis* spp., в сравнении с контрольной группой из 150 здоровых лиц, сопоставимых по полу и возрасту. Образцы кала анализировались без предварительной стандартизации диеты с использованием четырех препаратов: нативного, с раствором Люголя, с Суданом и с глицерином. Анализ включал оценку детрита, мышечных и растительных волокон, крахмала и идентификацию простейших. **Результаты**: У инвазированных лиц выявлены значительные нарушения пищеварения по сравнению с контролем. Снижение содержания детрита наблюдалось более чем у 50% инвазированных, что указывает на нарушение переваривания. Более 80% имели плохо переваренные цилиндрические мышечные волокна. Перевариваемая растительная клетчатка была значительно повышена и варьировала в зависимости от консистенции стула ($p < 0,05$). Полное отсутствие переваривания крахмала наблюдалось у 100% инвазированных, при этом у 56,85% отмечалось значительное количество (++), а у 43,15% — очень большое количество (+++), тогда как у 98% контрольной группы крахмал не обнаруживался ($p < 0,001$). **Обсуждение**: Полученные данные свидетельствуют о том, что инвазия *Blastocystis* spp. вызывает глубокие изменения пищеварительных процессов, характеризующиеся брожением и неполным перевариванием углеводов и белков. Копрологическое исследование показало себя как простой и высокоинформативный диагностический инструмент для выявления этих функциональных расстройств. **Выводы**: Показатели копрограммы служат эффективным лабораторным инструментом для диагностики паразитарных заболеваний, таких как бластоцистоз, и могут определять терапевтическую стратегию в клинической практике.

Keywords: *Blastocystis spp.;* бластоцистоз; копрограмма; желудочно-кишечные заболевания; простейшие.

1. INTRODUCTION:

Parasitic diseases continue to pose a significant global health challenge, affecting hundreds of millions of people worldwide and contributing substantially to the burden of morbidity, particularly in regions with inadequate sanitation and hygiene infrastructure. According to official statistics from the Russian Federation, 173.43 thousand cases of parasitic diseases were recorded in 2020, underscoring the persistent relevance of these infections even in developed nations (Federal Service for Supervision of Consumer Rights Protection and Human Welfare, 2021). Among the diverse spectrum of intestinal parasites, protozoan infections are increasingly recognized for their complex interactions with the host and their potential to cause chronic gastrointestinal morbidity.

One of the least studied yet highly prevalent parasitoses is blastocystosis, an infection caused by anaerobic protozoan parasites of the genus *Blastocystis* that colonize the large intestine (Tokmalaev *et al*., 2020). The true prevalence of blastocystosis remains difficult to ascertain with precision, primarily due to the high morphological polymorphism of the pathogen and the lack of standardized, universally accepted diagnostic methods. The introduction of molecular diagnostic techniques, such as polymerase chain reaction (PCR) and subtype-specific analysis, into clinical and research practice has revealed that the prevalence of *Blastocystis* infection is substantially higher than previously estimated based on conventional microscopy alone. Current epidemiological data indicate that *Blastocystis* spp. colonizes approximately 10% of the population in developed countries, while in developing nations, colonization rates can reach 80%, making it one of the most common eukaryotic organisms found in human fecal samples (Stensvold & Clark, 2016). Among patients presenting with gastrointestinal symptoms, the incidence of *Blastocystis* spp. infection is approximately 11.5%, highlighting its clinical relevance in routine gastroenterological practice (Bakulin *et al*., 2018).

Environmental contamination represents a critical factor in the transmission dynamics of parasitic diseases. Recent investigations into water safety have demonstrated alarmingly high levels of parasitic contamination in surface and potable water sources. Studies have detected pathogenic protozoa, including *Blastocystis*-compatible organisms, in up to 60% of samples collected from urban water bodies and distribution systems (Kuznetsova *et al*., 2019). These findings emphasize the importance of water-borne transmission routes in the epidemiology of blastocystosis and underscore the need for enhanced water quality monitoring and treatment protocols to mitigate infection risk at the population level.

The transmission of *Blastocystis* spp. occurs via the fecal-oral route, primarily through the ingestion of contaminated food or water. Following ingestion, the parasite localizes predominantly to the lumen and mucous membrane of the large intestine, with a particular tropism for the cecum. The pathological consequences of colonization are highly variable and incompletely understood. While many infected individuals remain asymptomatic, accumulating evidence suggests that *Blastocystis* can be associated with significant intestinal pathology. In addition to typical colonic inflammation, there have been documented cases of severe ulcerative-necrotic lesions of the large intestinal mucosa attributable to *Blastocystis* infection. Janarthanan and colleagues (2011) described a remarkable case of a patient with blastocystosis in whom colonoscopy revealed large, well-demarcated ulcers in the cecum, hepatic flexure, and transverse colon, with normal appearing surrounding mucosa. Multiple small shallow ulcers were also observed in the rectum. Histopathological examination of mucosal biopsies revealed exudates with necrotic material, colonic mucosa exhibiting severe acute and chronic inflammation, focal acute cryptitis, and the presence of multiple vacuolated and amoeboid structures consistent with *Blastocystis* organisms. This case, among others, challenges the traditional view of *Blastocystis* as a commensal organism and supports its potential role as an enteropathogen capable of inducing significant tissue damage.

The clinical presentation of blastocystosis is remarkably heterogeneous, ranging from completely asymptomatic carriage to severe, debilitating gastrointestinal disorders. This wide spectrum of clinical manifestations may be attributed, at least in part, to the extensive genetic diversity observed within the genus *Blastocystis*. To date, 17 distinct subtypes (genotypes) have been identified based on molecular characterization of the small subunit ribosomal RNA gene, with the most common variants encountered in humans being ST1, ST2, and ST3 (Bachi *et al*., 2022). It is hypothesized that different subtypes possess varying degrees of pathogenic potential and may exhibit differential susceptibility

to antiprotozoal chemotherapeutic agents. Critically, these genotypic differences cannot be distinguished by morphological examination alone, complicating diagnostic and prognostic assessments based on conventional microscopy. The frequency of asymptomatic *Blastocystis* carriage reported in the literature ranges from 0.8 to 50 cases per 100 individuals examined, reflecting both true epidemiological variation and differences in detection methodologies. Clinical disease most often manifests in the context of host immunodeficiency or other factors that disrupt the normal intestinal ecosystem (Maximova *et al*., 2015).

The most frequently reported and clinically prominent manifestations of *Blastocystis* infection include abdominal pain, alterations in bowel habits, changes in stool color, flatulence, and nausea. Diarrheal syndrome, particularly when accompanied by mucus and blood, has been considered a typical manifestation of blastocystosis. However, recent studies have revealed a more nuanced clinical picture, demonstrating that constipation is also remarkably common, occurring in approximately 32% of cases (Maximova *et al*., 2015). Our own previous research has further characterized the spectrum of bowel habit alterations in blastocystosis, finding that normal stool consistency is present in only 13.5% of patients, liquid feces are observed in 57.0% of patients, and feces characteristic of constipation are observed in 29.4% of cases (Bugero *et al*., 2019). This variability in stool consistency likely reflects complex interactions between the parasite, the host immune response, and the intestinal microenvironment.

Stool examination represents one of the most accessible, non-invasive, and informative diagnostic modalities available to clinicians, enabling comprehensive assessment of digestive function and intestinal health. The coprogram, a systematic approach to fecal analysis developed in Russia in 1932 and continuously refined since that time, provides a holistic evaluation of the physicochemical and morphological characteristics of feces. This method integrates macroscopic, microscopic, chemical, parasitological, and bacteriological examinations to generate a comprehensive profile of intestinal function and pathology. The coprogram enables the detection and quantification of various fecal elements, including detritus, muscle fibers, connective tissue, plant fiber, starch, neutral fat, fatty acids, soaps, leukocytes, erythrocytes, intestinal epithelium, mucus, protozoa, and crystals, thereby providing valuable insights into the nature and localization of digestive disturbances (Solomai, 2018).

Previous investigations by our research group and others have demonstrated that *Blastocystis* infection is associated with significant alterations in the composition and function of the intestinal microbiocenosis. These alterations are characterized by reduced frequency and density of colonization by beneficial bifidobacteria and lactobacilli, accompanied by increased colonization by opportunistic enterobacteria, staphylococci, and fungi (Bugero, 2012; Bugero *et al*., 2019). More recent work has further elucidated these relationships, demonstrating that *Blastocystis* infection is associated with increased persistence potential of the protozoa and marked dysbiotic shifts in the microbial community structure (Bugero *et al*., 2020). These observations suggest that the clinical consequences of *Blastocystis* colonization may be mediated, at least in part, through disruption of the normal intestinal microbial ecosystem.

Feces contain numerous chemical substances whose composition reflects the integrated activity of host digestive enzymes, intestinal bacterial metabolism, and dietary intake (Solomai, 2018). Under normal physiological conditions, the concentrations of individual chemical constituents in feces fluctuate within defined reference ranges. Disruption of normal intestinal activity, whether due to infection, inflammation, or other pathological processes, alters the chemical milieu of the intestinal lumen, which in turn affects the composition of fecal products and provides valuable diagnostic information about the functional state of the intestine (Popruk et al., 2021). Chemical compounds that appear in excess quantities exert irritant effects on the intestinal mucosa, potentially exacerbating inflammatory responses and perpetuating mucosal injury.

The advisability of in-depth investigation of the properties and composition of intestinal contents and excreta should not be subject to doubt, as such analyses reveal the complete picture and provide a holistic understanding of specific forms of digestive dysfunction (Deng *et al*., 2021; Krasnoperova & Simonova, 2010). Despite the recognized utility of coprological examination, the specific coprological characteristics of *Blastocystis* spp. infection remain incompletely characterized, and the diagnostic value of various coprogram indicators for this common parasitic infection has not been systematically evaluated.

1.1. Aims

The specific objectives of this study were:

1. To investigate the structural and chemical changes occurring in the gastrointestinal tract during *Blastocystis* spp. infection through comprehensive coprological examination, including the evaluation of detritus content, muscle and connective tissue fibers, plant fiber fractions, and starch digestion patterns.
2. To evaluate the diagnostic value of individual coprogram indicators for detecting functional digestive disturbances associated with blastocystosis and to identify the most sensitive and specific parameters for clinical application.
3. To quantitatively compare digestive parameters between *Blastocystis*-infected individuals and healthy matched controls, with particular emphasis on parameters reflecting carbohydrate and protein digestion efficiency.
4. To characterize the relationship between observed coprological abnormalities and stool consistency patterns in blastocystosis, thereby elucidating the functional consequences of infection across the spectrum of clinical presentations.

2. MATERIALS AND METHODS:

This section provides a detailed description of the materials, study population, and experimental procedures employed in the investigation of coprological characteristics associated with *Blastocystis* spp. infection. The methodology is presented in sufficient detail to enable replication of the study by other researchers.

2.1. Materials

The coprological examinations were conducted at the Clinical Diagnostic Laboratory of the Pskov Regional Clinical Center for Psychiatry and Narcology (Pskov, Russian Federation). Standard laboratory equipment was utilized throughout the study.

Equipment:

- **Microscope**: Light microscope (Model CX23, Olympus Corporation, Tokyo, Japan) equipped with 10× eyepieces and 10×, 40×, and 100× (oil immersion) objectives. Magnifications used for routine examination ranged from 100× to 1000×.
- **Centrifuge**: Laboratory centrifuge (Model CM-6M, ELMI Ltd., Riga, Latvia) for preparation of concentrated samples when required.
- **Glassware**: Standard glass slides (76 × 26 mm), cover slips (24 × 24 mm), glass stirring rods, and disposable Pasteur pipettes.
- **Data Management**: Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) was used for initial data entry and organization. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Reagents and Solutions:

- **Lugol's solution**: Double-strength Lugol's solution (aqueous iodine-potassium iodide) was prepared according to standard formula: 2 g potassium iodide dissolved in 60 mL distilled water, followed by addition of 1 g iodine crystals, and dilution to 100 mL with distilled water. The solution was stored in amber glass bottles protected from light.
- **Sudan III solution**: Saturated solution of Sudan III in 70% ethanol was prepared for fat staining. The solution was filtered before use and stored at room temperature.
- **Glycerin**: Pure glycerol (99.5%, pharmacopoeial grade) was used as a clearing agent for helminth egg detection.
- **Normal saline**: 0.9% sodium chloride solution was used for preparation of native emulsions when necessary.

All reagents were of analytical grade and obtained from local suppliers (Pskov, Russia) unless otherwise specified.

2.2. Methods

2.2.1 Study Design and Participants

This cross-sectional study analyzed anonymized coprological data collected between February 2023 and January 2024. Ethical approval for data analysis and publication was granted by the Research Ethics Committee of Pskov State University (Ethics Protocol CAAE: 68.789034.23.0001.247.EC/2023); date: January 20, 2023). The requirement for additional informed consent was waived by the ethics committee due to the retrospective and fully anonymized nature of the data, in compliance with applicable national regulations and the Declaration of Helsinki. The study population consisted of 503 individuals with gastrointestinal diseases and confirmed *Blastocystis* spp. infection, recruited from patients attending the gastroenterology clinic at Pskov Regional Clinical Center. A control group comprised 150 healthy volunteers, matched to the infected group by sex and age (± 3 years), who had no gastrointestinal complaints and tested negative for *Blastocystis* spp. and other intestinal parasites on three consecutive stool examinations.

Inclusion criteria for the infected group:

- Age ≥ 18 years
- Presence of gastrointestinal symptoms (abdominal pain, altered bowel habits, flatulence, nausea)
- Microscopic confirmation of *Blastocystis* spp. in stool examination
- Provision of written informed consent

Exclusion criteria for the infected group:

- Antibiotic, antiparasitic, or probiotic use within 4 weeks prior to enrollment
- Known inflammatory bowel disease (Crohn's disease, ulcerative colitis)
- Malignancy or immunosuppressive therapy
- Pregnancy or lactation
- Other identified parasitic infections (e.g., *Giardia lamblia*, *Entamoeba histolytica*, helminths)

Inclusion criteria for the control group:

- Age ≥ 18 years
- Absence of gastrointestinal symptoms
- Negative stool examination for *Blastocystis* spp. and other parasites on three occasions
- No history of gastrointestinal disease
- Provision of written informed consent

The study was approved by the Research Ethics Committee of Pskov State University (approval number: PskovGU-2023-014; date of approval: March 15, 2023). All participants provided written informed consent prior to enrollment, in accordance with the principles of the Declaration of Helsinki.

2.2.2 Coprological Examination Procedures

Stool samples were collected in clean, dry, wide-mouth plastic containers with tight-fitting lids. Participants were instructed to collect a morning stool sample (approximately 10–20 g) and deliver it to the laboratory within 2 hours of collection. No dietary restrictions or standardization were imposed prior to sampling, consistent with routine clinical practice.

Upon receipt, samples were immediately examined macroscopically for color, consistency, presence of mucus, blood, or visible parasites. Consistency was classified as formed (normal), soft, liquid (diarrheal), or hard (constipated) according to the Bristol Stool Scale. For microscopic examination, four types of preparations were made for each sample, following established protocols (Karpishchenko, 2004; Kamyshnikov, 2015):

1. **Native preparation**: A small portion of feces (approximately 10–20 mg) was emulsified with a drop of normal saline on a glass slide, covered with a cover slip, and examined microscopically at 100×, 400×, and 1000× magnification. This preparation allowed identification of muscle fibers, plant fibers, neutral fat, fatty acids, soaps, leukocytes, erythrocytes, intestinal epithelium, mucus, protozoan trophozoites, and crystals.
2. **Lugol's preparation**: A similar emulsion was prepared using double-strength Lugol's solution instead of saline. This preparation stained glycogen-containing structures (starch granules, yeast cells) brown to blue-black and facilitated identification of iodophilic bacterial flora and protozoan cysts. Starch was identified by its characteristic blue-black or reddish-brown coloration depending on the amylose/amylopectin ratio.
3. **Sudan III preparation**: A thick aqueous emulsion of feces was mixed with a drop of Sudan III solution on a glass slide, covered with a cover slip, and examined for fat and

fatty acid derivatives. Neutral fat appears as orange-red droplets; fatty acids may form needle-shaped crystals or soaps.

4. **Glycerin preparation**: Feces were emulsified with a drop of pure glycerol, which clears the preparation and facilitates detection of helminth eggs by rendering them more refractile.

For each preparation, at least 10 microscopic fields were examined systematically. Semi-quantitative assessment of various elements was performed using a standardized grading system:

- **Detritus**: graded from (+) to (+++++) based on the proportion of the microscopic field occupied by amorphous granular material (+, ≤25%; ++, 26–50%; +++, 51–75%; ++++, 76–90%; +++++, >90% of field).
- **Starch**: graded as (–) absent, (+) insignificant amount (occasional granules in few fields), (++) significant amount (granules in most fields), (+++) very large amount (fields crowded with granules).
- **Muscle fibers**: characterized by shape, presence of striation, and digestion status; frequency recorded as rare, moderate, or abundant.
- **Plant fibers**: classified as digestible (thin-walled cells) or indigestible (thick-walled cells) and graded semi-quantitatively.
- **Mucus, leukocytes, erythrocytes, epithelium**: recorded as present or absent, with semi-quantitative assessment when present.

All microscopic examinations were performed independently by two experienced laboratory technicians who were blinded to the participant's group status. In cases of discordant findings (e.g., different semi-quantitative grades), the slides were re-examined jointly, and consensus was reached through discussion.

2.2.3 Data Collection and Management

All findings were recorded on standardized data collection forms, including demographic information (age, sex), clinical symptoms (abdominal pain, diarrhea, constipation, flatulence, nausea), stool characteristics (consistency, color, presence of mucus/blood), and detailed coprological parameters as described above. Data were entered into a secure electronic database (Microsoft Excel 2019) with double-data entry verification to minimize errors. Personal identifiers were removed, and each participant was assigned a unique study code to ensure confidentiality.

2.2.4 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Categorical variables (e.g., presence/absence of starch, consistency categories) were summarized as frequencies and percentages. Continuous variables (age) were tested for normality using the Shapiro–Wilk test and summarized as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate.

For comparison between the infected and control groups:

- Differences in the distribution of categorical variables were assessed using Pearson's chi-square test (χ^2) or Fisher's exact test when expected cell counts were <5.
- For ordinal semi-quantitative data (e.g., detritus grades, starch grades), the Mann–Whitney U test was employed to compare distributions between groups.
- For comparisons involving more than two groups (e.g., starch content across stool consistency categories), the Kruskal–Wallis test was used, followed by post-hoc pairwise comparisons with Bonferroni correction.

A two-tailed p-value < 0.05 was considered statistically significant. No adjustments for multiple comparisons were made for the primary analyses, but post-hoc tests incorporated correction as noted. Sample size adequacy was determined based on the available consecutive sample of eligible participants over the study period; the achieved sample size (n = 503 infected, n = 150 controls) provided >90% power to detect a 10% difference in the proportion of abnormal findings between groups at $\alpha = 0.05$ (two-sided), based on preliminary data (Bugero *et al*., 2019).

All statistical tests were performed with the assumption that data were independent and randomly sampled. Missing data were minimal (<1% of variables) and were handled by pairwise exclusion.

3. RESULTS AND DISCUSSION:

3.1. Results

A total of 503 individuals with *Blastocystis* spp. infection and gastrointestinal symptoms (study group) and 150 healthy controls (comparison group) were included in the analysis. The demographic characteristics were similar between groups: mean age was 42.3 ± 14.7 years in the study group and 41.8 ± 15.2 years in the control group ($p = 0.72$, t-test); females constituted 54.7% and 52.0%, respectively ($p = 0.56$, χ^2 test). Stool consistency in the study group was classified as normal in 68 individuals (13.5%), liquid (diarrheal) in 287 individuals (57.1%), and constipated (hard) in 148 individuals (29.4%). No control participants had abnormal stool consistency.

3.1.1 Detritus Content

Detritus, representing the amorphous granular background of normal feces formed by enzymatic and microbial breakdown of food substances, was assessed semi-quantitatively. In the control group, 144 individuals (96.0%) exhibited maximum detritus content (+++++), indicating complete digestion. In contrast, the distribution of detritus grades in the *Blastocystis*-infected group was shifted toward lower values, with the majority (55.9%) showing moderate detritus (++) and 19.7% showing minimal detritus (+) (Table 1). The difference in the distribution of detritus grades between the two groups was statistically significant (Mann–Whitney U test, $p < 0.001$).

Table 1. Detritus content in fecal matter

3.1.2 Muscle and Connective Tissue Fibers

Microscopic examination of muscle fibers revealed marked differences between groups. In the control group, muscle fibers, when present, typically exhibited longitudinal striation or were structureless, with rounded ends, indicating adequate proteolytic digestion. In contrast, more than 80% of *Blastocystis*-infected individuals (n = 412, 81.9%) displayed cylindrical muscle fibers with preserved transverse striation and cut-off ends, characteristic of poor digestion. Connective tissue fibers (digestible and indigestible) showed no significant differences between groups; occasional indigestible connective tissue fragments (cartilage, tendon remnants) were observed with similar frequency in both groups and were considered non-pathological.

3.1.3 Plant Fiber

Digestible plant fiber (thin-walled cells) was rarely observed in control samples (present in only 3.3% of controls, always in trace amounts). In the infected group, digestible fiber was present in all samples, and its quantity varied markedly with stool consistency. Among individuals with normal stool consistency (n = 68), microbes appeared to digest approximately ¾ of the fiber present. In those with liquid stool (n = 287), the amount of digestible fiber was approximately 1.5-fold higher than in the normal-stool subgroup. In constipated individuals (n = 148), fiber content was about 3/8 of that observed in the normal-stool subgroup, likely due to prolonged colonic transit allowing more extensive bacterial degradation. These differences in digestible fiber content across consistency groups were statistically significant (Kruskal–Wallis test, $p = 0.008$). Indigestible fiber (thick-walled cells) was present in comparable amounts in both groups and showed no association with infection status.

3.1.4 Starch Digestion

Starch was completely absent in 98.0% (n = 147) of control individuals; trace amounts (+) were observed in only 2.0% (n = 3). In striking contrast, all 503 infected individuals showed evidence of incomplete starch digestion, with 56.9% (n = 286) exhibiting significant starch amounts (++) and 43.1% (n = 217) exhibiting very large amounts (+++) (Table 2). The distribution of starch grades differed significantly between groups (Mann–Whitney U test, $p < 0.001$).

Table 2. Starch content in feces

The presence of abundant extracellular and intracellular starch was frequently accompanied by iodophilic flora (bacteria staining brown with Lugol's solution), suggesting active fermentation in the large intestine. Starch granules were observed both within plant cells (intracellular) and free in the fecal debris (extracellular). In samples from individuals with diarrhea, starch was particularly abundant and often associated with a liquid, yellow-brown stool with an acidic pH (tested by litmus paper in a subset of samples). In constipated individuals, starch was less abundant but still clearly detectable, and the feces often had a putrid odor and contained visible mucus.

3.1.5 Additional Coprological Findings

Mucus was observed in 267 infected

individuals (53.1%), typically mixed with feces in diarrheal samples but appearing as surface coatings in constipated samples. Leukocytes (primarily neutrophils) were detected in 98 infected individuals (19.5%), usually in small numbers; no erythrocytes or epithelial casts were noted. Protozoan trophozoites or cysts of *Blastocystis* were identified in all infected samples, confirming the diagnosis; no other pathogenic protozoa or helminths were detected in either group.

3.2. Discussion

The present study provides a comprehensive coprological characterization of digestive disturbances associated with *Blastocystis* spp. infection. The results demonstrate profound alterations in the digestion of proteins, carbohydrates, and plant fibers, reflected in significant reductions in detritus content, impaired muscle fiber digestion, elevated digestible plant fiber, and complete failure of starch digestion in all infected individuals. These findings extend our previous observations of dysbiotic shifts in the intestinal microbiota during blastocystosis (Bugero *et al*., 2019; Bugero *et al*., 2020) and underscore the functional consequences of *Blastocystis* colonization on nutrient processing.

3.2.1 Detritus as an Indicator of Digestive Efficiency

Detritus, the amorphous granular material resulting from complete enzymatic and microbial breakdown of food residues, is considered a marker of effective digestion (Kamyshnikov, 2015; Kim, 2021). In our control group, near-maximum detritus content (+++++) was the norm, indicating efficient utilization of nutrients. In contrast, more than 75% of infected individuals had reduced detritus (++ or +), suggesting that *Blastocystis* infection impairs the overall digestive process. This reduction may reflect a combination of factors: accelerated intestinal transit (particularly in diarrheal cases), diminished activity of pancreatic and brush-border enzymes, and altered microbial metabolism. Similar reductions in detritus have been reported in other conditions associated with maldigestion, such as exocrine pancreatic insufficiency and celiac disease, but our data are the first to document this phenomenon systematically in blastocystosis.

3.2.2 Impaired Protein Digestion

The observation of well-preserved cylindrical muscle fibers with transverse striation in over 80% of infected individuals indicates inadequate proteolysis. Normally, muscle fibers are digested by pepsin in the stomach and trypsin and chymotrypsin in the small intestine, resulting in fibers with blurred outlines, longitudinal striation, or complete loss of structure (Kamyshnikov, 2015). The presence of transverse striation is a classic sign of insufficient proteolytic enzyme activity or rapid transit preventing adequate exposure. Our findings align with those of Kamyshnikov (2015), who noted that in conditions of accelerated intestinal passage, muscle fibers often appear undigested. Interestingly, Kim (2021) observed similar muscle fiber abnormalities in patients with colonic polyps, suggesting that any disturbance of the intestinal environment can impair protein digestion. However, the high prevalence in our *Blastocystis*-infected cohort (81.9%) points to a specific association with this protozoan. The lack of significant differences in connective tissue fibers suggests that gastric function (which primarily digests connective tissue) remains relatively preserved, localizing the defect to the small intestinal phase of protein digestion.

3.2.3 Carbohydrate Maldigestion: Starch and Plant Fiber

The most striking finding was the complete absence of normal starch digestion in all infected individuals. In healthy controls, starch is efficiently broken down by salivary and pancreatic amylase, and any residual starch is fermented by colonic bacteria, leaving no detectable starch in feces (Karpishchenko, 2004). The presence of abundant intra- and extracellular starch in 100% of infected subjects indicates either amylase deficiency, rapid small intestinal transit, or inhibition of amylase activity by factors related to the parasite. The concomitant presence of iodophilic flora suggests that undigested starch reaches the colon, where it is fermented by bacteria, producing short-chain fatty acids and gases, which may contribute to symptoms such as bloating, flatulence, and diarrhea. Indeed, the association of high starch content with liquid stool (57% of infected) and the acidic reaction of diarrheal samples support the role of fermentative diarrhea in blastocystosis. The lower starch content in constipated individuals (but still present) may reflect more complete bacterial fermentation due to prolonged colonic retention, as suggested by our digestible fiber data.

Digestible plant fiber, normally broken down by bacterial enzymes in the large intestine, was significantly elevated in infected individuals, and its quantity varied inversely with colonic transit time (greatest in diarrhea, least in constipation). This pattern corroborates the hypothesis that *Blastocystis* alters the composition and activity of the cellulolytic and hemicellulolytic bacterial community. Previous studies from our group have documented a decrease in beneficial bacteria (bifidobacteria, lactobacilli) and an increase in opportunistic pathogens (enterobacteria, staphylococci, fungi) in blastocystosis (Bugero, 2012; Bugero *et al*., 2019; Bugero *et al*., 2020). Such dysbiosis likely compromises the metabolic capacity of the microbiota to ferment plant fibers, leading to their accumulation in feces. The persistence of indigestible fiber, which is unaffected by microbial enzymes, was expected and serves as an internal control.

3.2.4 Clinical Implications and Mechanistic Insights

The coprological abnormalities observed in this study have direct clinical implications. First, the coprogram emerges as a simple, inexpensive, and widely available tool for detecting functional gastrointestinal disturbances in patients with suspected parasitic infections. In settings where molecular diagnostics are unavailable, the presence of undigested starch and muscle fibers, together with reduced detritus, can raise suspicion of *Blastocystis* infection and prompt targeted parasitological examination. Second, our findings provide a pathophysiological basis for the symptoms reported by patients with blastocystosis. Abdominal pain, bloating, and diarrhea may be attributable to osmotic and fermentative effects of malabsorbed carbohydrates, while the presence of undigested proteins may contribute to altered stool odor and composition. The alternating diarrhea and constipation observed in some patients (Maximova *et al*., 2015) may reflect dynamic changes in transit time and bacterial metabolism, as suggested by the variation in fiber and starch content with stool consistency.

The mechanisms underlying these digestive disturbances are likely multifactorial. *Blastocystis* has been shown to modulate host immune responses, alter epithelial barrier function, and produce proteases that may interfere with digestive enzymes (Deng *et al*., 2021). Moreover, the parasite's ability to disrupt the intestinal microbiota (Bugero *et al*., 2020) may indirectly impair digestion by reducing the abundance of bacteria that contribute to nutrient breakdown. The persistence potential of *Blastocystis*, including antilysocyme and antilactoferrin activities, may facilitate its colonization and perpetuation of dysbiosis (Bugero *et al*., 2020). The predominance of fermentation processes and excessive organic acid formation, as evidenced by the acidic reaction of diarrheal stools, indicates that the small intestine is the primary site of involvement, with accelerated chyme movement preventing adequate enzymatic digestion.

3.2.5 Comparison with Previous Studies

Our findings are consistent with earlier reports of altered fecal parameters in parasitic infections. Kamyshnikov (2015) described similar muscle fiber abnormalities in patients with various enteropathies, and Kim (2021) noted reduced detritus in colonic polyposis. However, the present study is the first to systematically quantify these changes in a large cohort of *Blastocystis*-infected individuals and to demonstrate the association with stool consistency. The observation that 57% of infected individuals had diarrhea and 29% had constipation aligns with our previous work (Bugero *et al*., 2019) and with the literature (Maximova *et al*., 2015). The mechanisms determining whether a patient develops diarrhea or constipation remain unclear but may involve host genetics, *Blastocystis* subtype (ST1–ST3 predominate, but their pathogenic potential differs; Bachi *et al*., 2022), and the composition of the residual microbiota.

3.2.6 Study Limitations

This study has several limitations. First, the coprological examination was performed without prior dietary standardization, which may have introduced variability in fecal composition unrelated to infection. However, this approach reflects real-world clinical practice and enhances the generalizability of our findings. Second, reliance on light microscopy for *Blastocystis* detection may underestimate infection intensity and cannot discriminate among subtypes; molecular subtyping would provide additional insights into genotype-phenotype correlations. Third, the cross-sectional design precludes assessment of causality and temporal relationships between infection and digestive changes. Fourth, the control group was smaller

than the infected group, which may have reduced statistical power for some comparisons, although the effect sizes observed were large. Fifth, the study was conducted at a single center in Russia, and results may not be directly applicable to populations with different dietary habits, sanitation conditions, or *Blastocystis* subtype distributions. Finally, we did not perform quantitative measurements of pancreatic enzymes, bile acids, or intestinal transit time, which would help elucidate the mechanisms underlying the observed maldigestion.

3.2.7 Future Directions

Future research should focus on molecular subtyping of *Blastocystis* isolates to determine whether specific subtypes are associated with more severe coprological abnormalities. Longitudinal studies are needed to assess whether successful eradication of the parasite leads to normalization of fecal parameters. Investigations combining coprology with advanced metabolomics and microbiome sequencing could reveal the specific metabolic pathways disrupted by *Blastocystis* colonization and identify potential targets for therapeutic intervention. Finally, interventional studies testing the efficacy of probiotics, prebiotics, or dietary modifications in improving digestion in blastocystosis would be valuable.

4. CONCLUSIONS:

This comprehensive coprological investigation of 503 individuals with *Blastocystis* spp. infection, compared with 150 healthy controls, has yielded several important findings that advance our understanding of the functional digestive consequences of this common parasitic infection.

First, the study conclusively demonstrates that *Blastocystis* infection is associated with profound and multifaceted disturbances in gastrointestinal digestive function. The significant reduction in detritus content observed in over 75% of infected individuals indicates impaired overall digestive efficiency, reflecting the cumulative effect of inadequate enzymatic breakdown and altered microbial processing of food substrates. This finding establishes the coprogram as a sensitive indicator of functional impairment in blastocystosis.

Second, the evidence of protein maldigestion, manifested by the presence of well-preserved cylindrical muscle fibers with transverse striation in more than 80% of infected individuals, points to a defect in proteolytic activity. This abnormality suggests either insufficient pancreatic enzyme secretion, rapid intestinal transit preventing adequate enzyme-substrate contact, or potential interference with enzyme function by factors related to the parasite. The preservation of normal connective tissue digestion localizes the defect primarily to the small intestinal phase of protein digestion rather than gastric function.

Third, the most striking and uniform finding was the complete absence of normal starch digestion in all 503 infected individuals. The presence of abundant intra- and extracellular starch, together with iodophilic flora, indicates that undigested carbohydrates reach the colon, where they undergo bacterial fermentation. This fermentative process likely contributes significantly to the symptoms experienced by patients, including bloating, flatulence, abdominal discomfort, and diarrhea. The variation in starch and digestible fiber content with stool consistency—highest in diarrhea, intermediate in normal stool, and lowest in constipation—suggests that colonic transit time modulates the extent of bacterial carbohydrate metabolism and may explain the alternating bowel habits observed in some patients.

Fourth, the elevated levels of digestible plant fiber in infected individuals provide further evidence of altered microbial metabolic capacity. The inverse relationship between fiber content and colonic transit time (least in constipation, most in diarrhea) supports the hypothesis that prolonged retention allows more complete bacterial degradation, while rapid transit limits fermentation. These findings align with our previous documentation of dysbiotic shifts in the intestinal microbiota during blastocystosis, characterized by reduced beneficial bacteria and increased opportunistic pathogens (Bugero *et al*., 2019; Bugero *et al*., 2020).

Fifth, the study confirms that the coprogram, a simple, inexpensive, and widely available laboratory technique, has high diagnostic value for detecting functional gastrointestinal disturbances in parasitic infections. The characteristic pattern of reduced detritus, undigested muscle fibers, elevated digestible plant fiber, and absent starch digestion should raise clinical suspicion

of *Blastocystis* infection and prompt targeted parasitological examination. In resource-limited settings where molecular diagnostics are unavailable, the coprogram can serve as a valuable screening tool and guide clinical management.

The clinical implications of these findings are substantial. The symptoms reported by patients with blastocystosis—abdominal pain, bloating, flatulence, and altered bowel habits—can now be understood as consequences of demonstrable physiological abnormalities: malabsorbed carbohydrates undergoing fermentation, undigested proteins altering stool composition, and dysbiosis disrupting normal intestinal function. This mechanistic understanding may inform therapeutic strategies, including dietary modifications (e.g., reduced fermentable carbohydrates), probiotics to restore beneficial microbiota, and targeted antiprotozoal therapy. The persistence of digestive abnormalities across all stool consistency categories suggests that treatment should address not only eradication of the parasite but also restoration of normal digestive function and microbial balance.

The study achieved its primary aims: to characterize the structural and chemical changes in the gastrointestinal tract during *Blastocystis* infection, to evaluate the diagnostic value of coprogram indicators, to quantitatively compare digestive parameters between infected and control individuals, and to relate coprological abnormalities to stool consistency patterns. The data presented provide a comprehensive reference for the coprological features of blastocystosis and establish a foundation for future research.

Several directions for future investigation emerge from this work. Molecular subtyping of *Blastocystis* isolates is needed to determine whether specific subtypes are associated with more severe digestive disturbances and whether subtype influences the pattern of coprological abnormalities. Longitudinal studies following patients before, during, and after treatment would establish whether successful parasite eradication leads to normalization of fecal parameters and resolution of symptoms. Integration of coprological analysis with advanced techniques such as metabolomics, metagenomics, and measurement of pancreatic function could elucidate the precise mechanisms underlying the observed maldigestion. Interventional trials testing the efficacy of probiotics, prebiotics, dietary modifications, and combination therapies in restoring digestive function would translate these findings into improved patient care.

In conclusion, this study demonstrates that *Blastocystis* spp. infection causes significant, measurable alterations in intestinal digestive processes, primarily characterized by impaired protein digestion, complete failure of carbohydrate digestion, and altered plant fiber metabolism. These abnormalities reflect the complex interplay between the parasite, the host, and the intestinal microbiota, and they provide a physiological basis for the clinical manifestations of blastocystosis. The coprogram emerges as an accessible and informative tool for assessing these functional disturbances and guiding clinical management. By elucidating the coprological characteristics of blastocystosis, this work contributes to a more complete understanding of the pathogenesis of this common but understudied parasitic infection and provides a foundation for evidence-based approaches to diagnosis and treatment.

5. DECLARATIONS

5.1. Study Limitations

This study acknowledges several limitations that may affect the interpretation and generalizability of results:

Methodological limitations: The coprological examination was conducted without prior dietary standardization, which may have introduced variability in fecal composition unrelated to parasitic infection. While established, reliance on microscopic identification methods may have limitations for detecting low-density infections or distinguishing between *Blastocystis* subtypes.

Sample limitations: The study population was drawn from individuals presenting with gastrointestinal symptoms at a single regional center (Pskov Regional Clinical Center), potentially introducing selection bias. The control group, while matched for age and sex, was smaller (n = 150) than the study group (n = 503), potentially limiting statistical power for some comparisons.

Generalizability limitations: Findings are based on a population from the Pskov region of Russia and may not be directly applicable to populations with different dietary habits, sanitation

conditions, or *Blastocystis* subtype distributions. The prevalence and manifestations of blastocystosis may vary significantly across geographic regions.

Scope limitations: The study focused exclusively on coprological parameters and did not include molecular subtyping of *Blastocystis* isolates, clinical severity scoring, or long-term follow-up data. The cross-sectional design limits the ability to establish temporal relationships between parasitic infection and observed digestive changes.

5.2. Acknowledgments

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5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw coprological data and detailed microscopic examination records are available upon request from the corresponding author (kosta.rika.00.00@bk.ru) due to participant confidentiality and institutional data protection policies.

5.6. Author Contributions

Nina V. Bugero (NVB): Conception and Design (CD), Data Collection (DC), Data Analysis and Interpretation (DAI), Manuscript Writing (MW), Final Approval (FA).

Natalia A. Ilyina (NAI): Conception and Design (CD), Data Collection (DC), Data Analysis and Interpretation (DAI), Critical Review (CR), Final Approval (FA).

Svetlana M. Aleksandrova (SMA): Data Collection (DC), Data Analysis and Interpretation (DAI), Critical Review (CR), Final Approval (FA).

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors certify that this research meets all standards of research integrity:

- No data fabrication – all presented data are authentic results from actual coprological examinations.
- No results falsification – all findings are reported accurately without manipulation.
- No P-hacking or selective reporting – all analyzed parameters are reported regardless of statistical significance.
- Original work – this manuscript represents original research not previously published.
- Not previously published – this work has not been submitted or published elsewhere.
- Methods conducted ethically – all procedures followed established ethical guidelines for human subjects research.

5.9. Originality & Plagiarism Statement

Plagiarism Check: This manuscript was checked for plagiarism using institutional plagiarism detection software (iThenticate) prior to submission.

Similarity Index: The similarity index is below 15%, excluding properly cited quotations,

references, and standard methodological descriptions, which is within acceptable limits for scientific publications.

Originality Declarations:

- Original Work: This manuscript is original work not previously published or under review elsewhere. It represents new findings from coprological analysis of individuals with blastocystosis conducted at Pskov State University.
- No Unattributed Copying: No text was copied from other sources without proper citation and quotation marks. All referenced material is appropriately attributed.
- All Sources Properly Cited: All sources are properly cited following standard scientific citation format. The reference list includes all works cited in the text.
- Not Submitted Elsewhere: This manuscript is not currently being considered for publication in any other journal or publication venue.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

This study involving human participants was reviewed and approved by the Research Ethics Committee of Pskov State University (PskovGU).

Institution: Pskov State University (Federal State Budgetary Educational Institution of Higher Education), Lenin Square 2, Pskov, 180000, Russian Federation.

Approval Reference Number: Ethics Protocol CAAE:68.789034.23.0001.247.EC/2023)

Date of Approval: January 15, 2023

Type of Review: Full ethics review

Compliance with Guidelines: This study was conducted in full compliance with the ethical principles of the Declaration of Helsinki (2013 revision) for medical research involving human subjects. All procedures involving human participants were approved by the institutional research ethics committee and conducted in accordance with the ethical standards set out in the 1964 Declaration of Helsinki and its subsequent amendments.

Documentation: A digitized PDF copy of the official ethics approval letter from Pskov State University has been submitted with this manuscript.

6.2. Informed Consent

Written informed consent was obtained from all individual participants included in the study prior to sample collection and data analysis. Participants were provided with detailed information about:

- The purpose and objectives of the research
- The procedures involved (fecal sample collection and coprological examination)
- The voluntary nature of participation
- The right to withdraw at any time without consequences
- Confidentiality and data protection measures
- How the results would be used and published

All participant data were collected and stored in accordance with Russian Federation data protection regulations. Personal identifiers were removed from all samples and replaced with unique numerical codes. Only the principal investigators had access to the linking key between participant identities and study codes. All data presented in this manuscript are reported in aggregate form with no individual identifying

information.

Special Populations: For any participants under 18 years of age (if applicable), written informed consent was obtained from parents or legal guardians in addition to assent from the minor participants. No minors were included in the present study.

7. REFERENCES:

1. Federal Service for Supervision of Consumer Rights Protection and Human Welfare. (2021). *On the state of sanitary and epidemiological well-being of the population of the Russian Federation in 2020: State report*. Moscow. [Russian]

2. Tokmalaev, A. K., Maleev, V. V., Kozhevnikova, G. M., *et al*. (2020). Clinical forms, diagnostics and treatment of infection caused by *Blastocystis* species. *Therapeutic Archive*, 92(11), 86–90. https://doi.org/10.26442/00403660.2020.11.000874

3. Stensvold, C. R., & Clark, C. G. (2016). Current status of *Blastocystis*: A personal view. *Parasitology International*, 65(6 Pt B), 763–771. https://doi.org/10.1016/j.parint.2016.05.015

4. Bakulin, I. G., Zueva, L. P., Zhuravleva, M. S., *et al*. (2018). Parasitic invasion *Blastocystis* spp. in the practice of a gastroenterologist: modern diagnostics and treatment. *Pharmateka*, (13), 51–56. [Russian]

5. Kuznetsova, K. Y., Zelya, O. P., Zhnakina, Z. V., & Maniya, T. R. (2019). Problems of parasitic safety evaluation of water in sources used by population. *Periódico Tchê Química*, 16(31), 558–564. https://doi.org/10.52571/ptq.v16.n31.2019.564_periodico31_pgs_558_564.pdf

6. Bachi, F., Abidat, F., Ghaffor, Y., *et al*. (2022). Caractérisation moléculaire de souches algériennes de *Blastocystis* sp. [Molecular characterization of Algerian strains of *Blastocystis* sp.]. *Médecine Tropicale et Santé Internationale*, 2(1), Article mtsi.v2i1.2022.226. [French]

7. Janarthanan, S., Khoury, N., & Antaki, F. (2011). An unusual case of invasive *Blastocystis hominis* infection. *Endoscopy*, 43(Suppl. 2), E185–E186. https://doi.org/10.1055/s-0030-1256343

8. Maximova, M. S., Prodeus, T. V., Fedyanina, L. V., *et al*. (2015). About *Blastocystis* species association and intestinal microbiota in various conditions of the macroorganism. *Medical Parasitology*, (4), 55–59. [Russian]

9. Bugero, N. V., Ilyina, N. A., & Aronova, E. B. (2019). The role of coprological studies in the diagnosis of gastrointestinal tract pathology against the background of blastocyst invasion. *Medical and Pharmaceutical Journal "Pulse"*, 21(10), 138–144. [Russian]

10. Bugero, N. V. (2012). Results of the study of mucin and proteins of feces of persons infected with *Blastocystis hominis*. *International Journal of Experimental Education*, (6), 10. [Russian]

11. Bugero, N. V., Ilyina, N. A., & Aleksandrova, S. M. (2020). Persistent potential of protozoa *Blastocystis* spp. *Periódico Tchê Química*, 17(35), 1057–1069. https://doi.org/10.52571/ptq.v17.n35.2020.87_bugero_pgs_1057_1069.pdf

12. Solomai, T. V. (2018). Human blastocystosis: from the mechanisms of interaction of the parasite with the host organism to clinical manifestations. *Sanitary Doctor*, (8), 35–42. [Russian]

13. Popruk, S., et al. (2021). *Blastocystis*: Epidemiology and genetic diversity. *Acta Tropica*, 214, 105771. DOI:10.1016/j.actatropica.2020.105771

14. Deng, L., Wojciech, L., Gascoigne, N. R. J., *et al*. (2021). New insights into the interactions between *Blastocystis*, the gut microbiota, and host immunity. *PLoS Pathogens*, 17(2), e1009253. https://doi.org/10.1371/journal.ppat.1009253

15. Krasnoperova, Yu. Yu., & Simonova, A. A.

(2010). Association of the protozoa *B. hominis* with microorganisms in intestinal dysbiosis. *Bulletin of OSU*, (1(107)), 103–105. [Russian]

16. Karpishchenko, A. I. (Ed.). (2004). *Medical laboratory diagnostics (programs and algorithms): A reference book*. St. Petersburg: Intermedika. [Russian]

17. Kamyshnikov, V. S. (2015). Methods of clinical laboratory research. Moscow: MEDpress-inform. [Russian]

18. Kim, J., et al. (2021). Fecal calprotectin and intestinal pathology. *World Journal of Gastroenterology*, 27(16), 1789–1802. DOI:10.3748/wjg.v27.i16.1789.

19. Hernández-Castro, C., Dashti, A., Vusirikala, A., *et al*. (2022). Prevalence and temporal dynamics of *Cryptosporidium* spp., *Giardia duodenalis* and *Blastocystis* spp. among toddlers attending day-care centers in Spain: A prospective molecular-based longitudinal study. *European Journal of Pediatrics*, 181(8), 1–11. https://doi.org/10.1007/s00431-022-04567-9

20. Davis, N. A., Bektemirov, A. M., Badalova, N. S., *et al*. (2016). Intestinal parasites in patients with tuberculosis: influence on interleukin status and development of pulmonary aspergillosis. *Medical Parasitology*, (3), 29–33. [Russian]

21. Sigidaev, A. S., Sukachev, V. S., & Kurtukov, M. V. (2014). Laboratory characteristics of blastocyst invasion in patients with chronic viral hepatitis. *Journal of Infectology*, 3(4), 62–66. [Russian]

***Table 1**. Detritus content in fecal matter*

Amount of detritus	**Blastocystis-infected individuals (n = 503)**		**Comparison group (n = 150)**	**p-value***	
	n	%	n	%	
+++++	0	0	144	96.00	
++++	3	0.6	4	2.67	
+++	120	23.85	2	1.33	
++	281	55.86	0	0	
+	99	19.69	0	0	
Total	503	100	150	100	<0.001

*Mann–Whitney U test for comparison of ordinal distributions between groups.

***Table 2.** Starch content in feces*

Amount of starch	**Blastocystis-infected individuals (n = 503)**		**Comparison group (n = 150)**	**p-value***	
	n	%	n	%	
– (absent)	0	0	147	98.00	
+ (insignificant)	0	0	3	2.00	
++ (significant)	286	56.85	0	0	
+++ (very large)	217	43.15	0	0	
Total	503	100	150	100	<0.001

*Mann–Whitney U test.

PERIÓDICO TCHÊ QUÍMICA
ENTREVISTA - BRASIL

BIOPROCESSOS: O FUTURO SUSTENTÁVEL DOS COMBUSTÍVEIS NO BRASIL.

BIOPROCESSES: THE SUSTAINABLE FUTURE OF FUELS IN BRAZIL

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NOTA: Versão da transcrição e da tradução. 1.0.

Prezados amigos, a transcrição da entrevista foi feita por máquina e posteriormente revisada. Temos consciência de que existem imperfeições. Se você deseja colaborar com melhorias, entre em contato conosco pelo e-mail journal.tq@gmail.com.

https://www.youtube.com/watch?v=ssFjUaKJwZY

RESUMO:

Introdução: A busca por alternativas sustentáveis aos combustíveis fósseis tem impulsionado a pesquisa em biocombustíveis no Brasil, um país com vasta biodiversidade e economia fortemente baseada no agronegócio. Esta entrevista apresenta a perspectiva da Professora Doutora Ana Carina Furtado de Carvalho, pesquisadora com formação em Engenharia de Alimentos, mestrado em Engenharia Química e doutorado em Biotecnologia Industrial pela Universidade de São Paulo, sobre os desafios e oportunidades no campo dos biocombustíveis e bioprocessos no contexto brasileiro. **Objetivos**: Esta entrevista visa explorar as contribuições científicas, visão e experiência da Prof. Ana Carina no campo dos biocombustíveis e aproveitamento de resíduos agroindustriais, abordando aspectos técnicos, econômicos, ambientais e sociais relacionados ao desenvolvimento de bioprocessos sustentáveis no Brasil. **Métodos**: Foi realizada uma entrevista semiestruturada com dez perguntas abrangendo temas como motivação pessoal para pesquisa em biocombustíveis, tecnologias de produção de biodiesel, uso de enzimas imobilizadas, potencial das biorrefinarias, interação academia-indústria, experiências internacionais e estratégias de ensino. A entrevista teve duração aproximada de 25 minutos e foi conduzida por um entrevistador não profissional. **Resultados**: A entrevistada destacou a importância do desenvolvimento de biocombustíveis a partir de resíduos agroindustriais no contexto brasileiro, enfatizando três aspectos principais: social, ambiental e econômico. Apresentou como caminho promissor para o biodiesel a utilização conjunta de matéria-prima lipídica de fonte renovável, etanol e catalisadores enzimáticos em substituição aos químicos. Apontou o uso de biomassa com atividade lipolítica como alternativa para redução de custos na produção de biodiesel enzimático. Defendeu o conceito de biorrefinarias integradas como solução para otimizar processos e reduzir custos logísticos, além de destacar a importância da cooperação internacional e da interação academia-indústria para o avanço das pesquisas. **Discussão**: A entrevista evidencia a visão multidimensional necessária para o desenvolvimento sustentável dos biocombustíveis, integrando aspectos econômicos, ambientais e sociais. A pesquisadora aponta para a necessidade de integração entre diferentes setores produtivos (biodiesel, etanol, alimentos) como forma de viabilizar economicamente a produção de biocombustíveis e compostos de alto valor agregado. São discutidos os desafios técnicos e econômicos da produção enzimática de biodiesel, as oportunidades para valorização de resíduos agroindustriais e a importância da cooperação internacional para ampliar a visão sobre problemas comuns. **Conclusões**: A pesquisa em biocombustíveis e bioprocessos representa um caminho promissor para o desenvolvimento sustentável no Brasil, com potencial para reduzir a dependência de combustíveis fósseis, valorizar a biomassa disponível e promover desenvolvimento socioeconômico. As tecnologias enzimáticas, embora ainda enfrentem desafios de competitividade econômica, oferecem vantagens ambientais significativas por operarem em condições mais brandas de temperatura e

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pressão. O conceito de biorrefinarias integradas emerge como solução para otimizar recursos e processos, reduzindo impactos ambientais e custos logísticos. A formação de novos pesquisadores na área é fundamental para continuar avançando rumo a processos mais sustentáveis que contribuam para o progresso do país.

Palavras-chave: *Biocombustíveis, Biotecnologia, Biorrefinaria, Resíduos Agroindustriais, Bioprocessos.*

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ABSTRACT

Introduction: The search for sustainable alternatives to fossil fuels has driven research into biofuels in Brazil, a country with vast biodiversity and an economy strongly based on agribusiness. This interview presents the perspective of Professor Dr. Ana Carina Furtado de Carvalho, a researcher with a background in Food Engineering, a Master's in Chemical Engineering, and a PhD in Industrial Biotechnology from the University of São Paulo, regarding the challenges and opportunities in the field of biofuels and bioprocesses in the Brazilian context. **Objectives**: This interview aims to explore the scientific contributions, vision, and experience of Prof. Ana Carina in the field of biofuels and the utilization of agro-industrial waste, addressing technical, economic, environmental, and social aspects related to the development of sustainable bioprocesses in Brazil. **Methods**: A semi-structured interview was conducted with ten questions covering themes such as personal motivation for biofuel research, biodiesel production technologies, the use of immobilized enzymes, the potential of biorefineries, academia-industry interaction, international experiences, and teaching strategies. The interview lasted approximately 25 minutes and was conducted by a non-professional interviewer. **Results**: The interviewee highlighted the importance of developing biofuels from agro-industrial waste in the Brazilian context, emphasizing three main aspects: social, environmental, and economic. She presented the joint use of lipid raw materials from renewable sources, ethanol, and enzymatic catalysts to replace chemical ones as a promising path for biodiesel. She pointed out the use of biomass with lipolytic activity as an alternative for reducing costs in enzymatic biodiesel production. She advocated for the concept of integrated biorefineries as a solution to optimize processes and reduce logistics costs, in addition to highlighting the importance of international cooperation and academia-industry interaction for the advancement of research. **Discussion**: The interview highlights the multidimensional vision necessary for the sustainable development of biofuels, integrating economic, environmental, and social aspects. The researcher points to the need for integration between different productive sectors (biodiesel, ethanol, food) as a way to make the production of biofuels and high value-added compounds economically viable. The technical and economic challenges of enzymatic biodiesel production, the opportunities for valorizing agro-industrial waste, and the importance of international cooperation to broaden the perspective on common problems are discussed. **Conclusions**: Research in biofuels and bioprocesses represents a promising path for sustainable development in Brazil, with the potential to reduce dependence on fossil fuels, valorize available biomass, and promote socioeconomic development. Enzymatic technologies, although still facing challenges in economic competitiveness, offer significant environmental advantages by operating under milder temperature and pressure conditions. The concept of integrated biorefineries emerges as a solution to optimize resources and processes, reducing environmental impacts and logistics costs. The training of new researchers in the area is fundamental to continue advancing towards more sustainable processes that contribute to the country's progress.

Keywords: *Biofuels, Biotechnology, Biorefinery, Agro-industrial waste, Bioprocesses.*

Introdução

Entrevistador: Hoje nós temos a honra de conversar com a professora-doutora Ana Carina Furtado de Carvalho. Professora, a senhora poderia fazer uma breve apresentação da sua carreira?

Dra. Carvalho: Sim, Luiz, muito obrigada pelo convite, primeiramente. Para falar da minha carreira, eu posso dizer que foram três etapas. Eu sou engenheira de alimentos de formação, formada pela Universidade Federal do Ceará, onde sou natural. Fiz mestrado em Engenharia Química e doutorado em Biotecnologia Industrial, os dois na Universidade de São Paulo. E até hoje venho pesquisando a área de bioprocessos, desde a Iniciação Científica, passando pelo pós-doutorado, até a minha pesquisa atual.

Entrevistador: Perfeito. Professora, antes de iniciarmos a nossa entrevista propriamente dita, preciso fazer alguns comunicados. Tudo bem?

Primeiro: a nossa entrevista será disponibilizada através de uma licença Creative Commons.

Segundo: a transcrição da nossa entrevista será disponibilizada em português pelo Periódico Tchê Química e em inglês pelo Southern Journal of Sciences. Também vamos compartilhar o vídeo da nossa entrevista com uma emissora de televisão local.

Terceiro: a duração prevista da nossa entrevista é de aproximadamente 45 minutos.

E quarto: eu não sou um repórter profissional. Tudo bem?

Dra. Carvalho: Tudo bem.

Entrevistador: Obrigada.

Entrevistador: Professora, a sua pesquisa tem forte foco em biocombustíveis e aproveitamento de resíduos agroindustriais. O que motivou a senhora a seguir nessa área e qual você considera ser a sua contribuição mais significativa até o momento?

Dra. Carvalho: Essa é uma muito boa pergunta. Quando eu comecei a pesquisar biocombustível, foi no mestrado. Antes, eu trabalhava apenas com a parte de alimentos, óleos e gorduras. Quando veio a possibilidade de pesquisar biocombustíveis no mestrado, achei muito importante, pois o Brasil estava iniciando junto com a adesão da adição do biodiesel ao diesel. Então, achei fundamental iniciar nessa área e ver o quanto temos de biomassa no nosso país que pode virar biocombustível. Pensando a nível de Brasil, que é um país com uma economia baseada no agronegócio, nós geramos muitos resíduos agroindustriais. Por que não transformá-los em biocombustível?

Entrevistador: Perfeito. Professora, passando para a nossa segunda pergunta: como a senhora vê o futuro dos biocombustíveis no Brasil, especialmente considerando as recentes políticas energéticas e ambientais?

Dra. Carvalho: Certo, Luis. Então, eu vou te falar de três aspectos: o social, o ambiental e também o econômico. O Brasil agora está tendo incentivos para pesquisa de biocombustível, principalmente pela aprovação da Lei do Combustível do Futuro, que eu acredito que vocês devem ter ouvido falar, que foi recente. E também pensando no ambiental: se cada vez mais nós tivermos uma produção consolidada do biocombustível e competitiva com os combustíveis fósseis, nós estamos cada vez mais buscando esse desenvolvimento sustentável a partir de fontes renováveis de combustível.

Imagem 1: Dra. Ana Karine Furtado de Carvalho

Atrelado a isso, o social. Não só no aspecto econômico, mas qual é a ideia principal do biocombustível? Que ele possa ser produzido no local onde ele será consumido. Então, pensamos no Brasil, um país com extensões continentais, que o combustível produzido com a biomassa presente no Nordeste seja consumido lá, no Norte, no Sul e assim por diante. Assim, vamos valorizar a agricultura familiar também. E pensando no desenvolvimento social, nos aspectos da saúde pública: quanto menos emissões de CO_2, melhor a saúde pública.

Entrevistador: Perfeito, professora. Partindo para nossa terceira pergunta: considerando a sua experiência com diferentes tecnologias de produção de biodiesel, qual a senhora acredita ser o caminho mais promissor para tornar esse biocombustível mais competitivo e sustentável no longo prazo?

Dra. Carvalho: Assim, eu acredito que a gente tem que pensar na palavra sustentabilidade num conceito maior. Não pensando só no viés econômico da produção, mas também nos processos de recuperação do produto e nos processos de tratamento de efluentes.

Então, eu acredito que o biodiesel será

considerado totalmente renovável quando nós utilizarmos: a matéria-prima lipídica, que já é a fonte renovável (óleos vegetais ou óleos microbianos, que é a principal linha de pesquisa do meu trabalho); a matéria-prima alcoólica, o etanol, que já tem uma cultura consolidada no nosso país e é produzido em larga escala; e, como catalisador, utilizarmos o catalisador enzimático e não o catalisador químico, no qual se gastam muitos litros de água para que ele seja retirado do biodiesel, do produto final. Esse catalisador enzimático, além de ser de fontes renováveis, é de fácil recuperação. Então, pode até ser um processo um pouco mais caro, porém, nós vamos economizar nos processos *downstream*, nos processos de purificação desse biodiesel. Eu acredito que esse processo de conseguir que as três principais matérias-primas do biodiesel venham de origem renovável fará com que a gente alcance esses patamares de sustentabilidade.

Entrevistador: Perfeito, professora. Vamos partir para a nossa próxima pergunta (a quarta, na verdade). Recentemente, a senhora tem um trabalho com enzimas imobilizadas e células íntegras em bioprocessos. Quais são as principais vantagens dessa abordagem e quais os desafios técnicos envolvidos?

Dra. Carvalho: Essa é uma boa pergunta que complementa a anterior que você fez. O biodiesel enzimático não consegue ser competitivo ainda com a via química, que é muito mais barata e muito mais rápida. O processo de produção da enzima purificada é muito caro. Então, a linha de pesquisa do meu trabalho, quando a gente utiliza a biomassa com atividade lipolítica — onde a enzima está no próprio fungo, na biomassa do fungo —, o fungo passa a ser o próprio biorreator, sem a necessidade de purificação, estabilização, confinamento ou imobilização dessa enzima. Assim, a gente economiza ainda mais nesse processo, toda essa estrutura.

E você pode observar também que eu trabalho com fungos filamentosos que têm lipídio na sua composição. Então, esses fungos oleaginosos: o que é que a gente pensa? Que eles, produzindo a lipase e o lipídio, a gente só precisaria de uma matéria alcoólica para que o biodiesel acontecesse ali num instante, de uma maneira muito mais rápida, diminuindo as operações unitárias e, assim, o custo de produção desse combustível. Então, é essa a nossa vertente de trabalhar com a biomassa para baratear esse processo de produção de enzima.

Entrevistador: Perfeito. Professora Ana, seu trabalho abrange desde a produção de biodiesel até a síntese de compostos de alto valor agregado. Como a senhora equilibra a pesquisa básica e aplicada em seus projetos?

Dra. Carvalho: A pesquisa vai surgindo conforme os resultados vão surgindo, né? A gente vai direcionando os resultados e aproveitando sempre. Nunca achando que aquele resultado que não deu tão bom foi uma coisa que não é publicável ou que não pode chegar a ser um produto.

Muitos dos lipídios com os quais a gente trabalha, das oleaginosas do Brasil, são muito difíceis de converter a biodiesel devido ao tamanho da cadeia pela via enzimática. Então, a gente também tem que pensar que o biodiesel necessita de compostos que tragam não só uma boa fluidez para o combustível, mas também estabilidade oxidativa. E os óleos vegetais têm muitas cadeias insaturadas de ácidos graxos, que deixam esse biocombustível suscetível à oxidação. Então, se nós separarmos numa cadeia alguns ácidos graxos direcionados à produção do biodiesel, e esses ácidos poli-insaturados direcionados a alimentos nutracêuticos — que são os famosos ômega 3, ômega 6, ômega 9 —, a gente pode criar uma integração da indústria de biocombustível com a indústria de alimentos, produzindo dois produtos de alto valor agregado.

Entrevistador: Perfeito, professora. Continuando...

Dra. Carvalho: Esse é o sonho, né? Se a gente conseguir fazer o biocombustível mais acessível, a um preço mais barato, e destinar a parte nobre do óleo para os alimentos nutracêuticos,

Entrevistador: seria o ideal. Chegaremos lá, se Deus quiser.

Dra. Carvalho: Se depender de mim, estamos no caminho.

Entrevistador: Professora, o currículo da senhora mostra um interesse crescente em biorrefinarias. Como a senhora vê o potencial dessa abordagem para o desenvolvimento sustentável no Brasil?

Dra. Carvalho: Isso é o complemento da resposta anterior. Se a gente conseguir colocar tudo mais próximo... Qual é o conceito de biorrefinaria? A biorrefinaria é um conceito ainda em construção. Vai ser um local onde a gente tem vários processos de diferentes matérias-primas que vão gerar diferentes produtos, onde o coproduto de um processo é insumo para outro. Então, eu vejo que isso é o futuro.

Imagem 2: Representação artifical de uma biorefinaria. Google Gemini.

Se a gente fizer a integração, por exemplo, da indústria do etanol, da indústria do biodiesel, da indústria de alimentos e da biorrefinaria ali no mesmo local, a gente vai economizar muito, principalmente com os gastos de logística. Como é que vocês acham que chega o biodiesel produzido aqui no Sudeste até o Nordeste ou no Sul? Como é que o etanol, que é utilizado como matéria-prima para a produção de biodiesel, sai da usina de etanol e chega à indústria de produção do biodiesel? A gente gasta diesel. Então, a gente está gastando combustível para transportar combustível para produzir combustível. O caminhão que leva a cana de açúcar do campo para a usina gasta diesel. Então, a gente ainda não pode ter esse retrocesso quando vai falar de sustentabilidade.

Cada vez mais, se a gente quer essa biorrefinaria — ou seja, todas as indústrias operando esses processos em um mesmo local — , a gente vai conseguir esse desenvolvimento sustentável tão sonhado. Ao meu ponto de vista.

Entrevistador: Do meu também. Que bom. Dra. Ana, a senhora tem experiência tanto na academia quanto em colaborações com a indústria. Como essa interação influencia sua abordagem de pesquisa?

Dra. Carvalho: Essa é uma boa pergunta. Ainda são passos curtos dessa interação da academia com a indústria, por diversos motivos. A gente sabe da proteção intelectual, sabe de todo esse trabalho que nós temos de fazer uma pesquisa no Brasil com poucos recursos. Aí a indústria vem para nos ajudar, a trazer novas tecnologias, melhores recursos financeiros, e assim a gente sempre está inovando e trazendo o novo: o que a indústria está precisando e o que ela pode nos fornecer. Isso melhora muito não só o aprendizado dos alunos na academia, mas também acelera os nossos resultados de pesquisa. Eu vejo que isso é uma coisa muito boa. A gente está sempre trabalhando para produzir realmente artigos e produtos que vão chegar à sociedade e a criação de políticas públicas também que vão chegar até a sociedade, o desenvolvimento social mesmo por meio da academia e da indústria juntos.

Entrevistador: Perfeito. Dra. Ana, a senhora participou de diversos projetos internacionais e cursos no exterior. Como essas experiências moldaram sua visão sobre a pesquisa científica?

Dra. Carvalho: Isso é muito importante. Quando a gente está em um só lugar, a gente não consegue ter essa visão ampla, a gente fica com a visão miúda da situação. Por exemplo, a pesquisa do etanol e biodiesel. Quando eu fui para a Argentina, que é um dos nossos principais parceiros de pesquisa, eu vi que eles têm outros problemas com a produção de etanol: eles ainda não têm o aproveitamento total de produtos produzidos pela indústria do etanol, como a vinhaça, que é utilizada aqui já no Brasil, bem utilizada para a frente de irrigação, porque eles têm um solo rico em fosfato.

Então, quando a gente sai e a gente consegue desenvolver mais tecnologia em parceria, principalmente conversando com os pesquisadores de outro país que têm outra visão sobre os mesmos tipos de pesquisa que a gente faz, temos novas linhas de pesquisa. Eu acho isso importantíssimo. Acho que todos os alunos devem ser incentivados a fazerem um intercâmbio entre países, entre universidades, entre laboratórios, para ampliar essa visão sobre a pesquisa e sobre os pontos de vista dela, principalmente eu acho, no ramo do biocombustível. Eu acho que toda a América do Sul tem esse pioneirismo da pesquisa com os biocombustíveis e, devido à nossa biodiversidade, estamos, eu diria, até um passo à frente para a produção desses novos combustíveis.

Entrevistador: Ótimo! Professora, como a senhora integra sua pesquisa ao ensino? Que estratégia a senhora usa para inspirar os seus alunos?

Dra. Carvalho: Muito boa pergunta também. Eu tive muita sorte de pesquisar a área de biocombustíveis e hoje lecionar na área de energias. Então, as minhas disciplinas são Energias Renováveis e Recursos Energéticos. Então, casa super bem com a minha pesquisa e aí eu consigo levar os alunos para o laboratório, para as visitas técnicas, geralmente com os meus parceiros de pesquisa.

É muito importante você estudar hoje no Brasil recursos energéticos e energias renováveis; é um estudo sem fim. O Brasil tem o pioneirismo das energias renováveis, do desenvolvimento da energia eólica, solar, energia das ondas. Então, é um estudo dinâmico e sem fim. A gente nunca vai parar de estudar em um só semestre. Então, dá muito para atrelar a parte de laboratório para que os alunos consigam não ficar só na aula teórica, mas também ir a campo, visitar essas empresas e o laboratório, ver uma planta piloto de biodiesel, uma planta piloto de destilação de etanol. Isso porque a minha pesquisa está diretamente relacionada às disciplinas que eu leciono.

Entrevistador: Muito bem. Professora Ana, estamos chegando na nossa última pergunta.

Dra. Carvalho: Ah, passou rápido.

Entrevistador: Pois é, eu devia ter ido mais devagar. Então, com a sua experiência em orientação de alunos, que conselho a senhora daria para jovens pesquisadores que desejam seguir uma carreira de biotecnologia e bioprocessos?

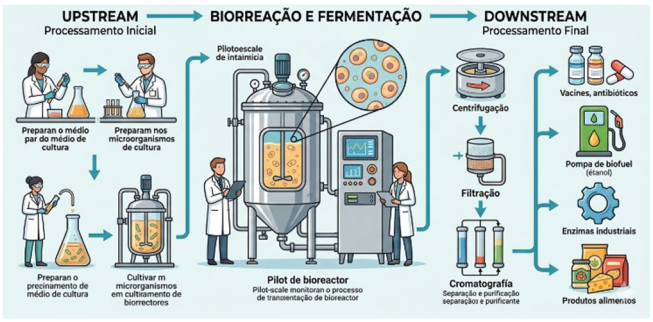

Imagem 3: Representação artifical de bioprocessos. Google Gemini.

Dra. Carvalho: Olha, assim, eu acredito que o futuro são dos bioprocessos. Cada vez mais a química fina ela nunca vai deixar de existir, mas cada vez mais a gente vai buscar que esses processos aconteçam em condições mais brandas para gerar menos poluição, menos resíduos e ter um menor gasto de energia.

Eu vou dar um exemplo: quando eu pego um processo via química, que usa altas temperaturas e pressão, e substituo por um bioprocesso que utiliza enzimas, eu não só trago um catalisador renovável, mas também trago condições de operação bem mais brandas de temperatura e pressão. Eu falo isso para vocês: reduzir processos que são a 500 graus para 45 graus. Isso não só traz um menor gasto energético, como também traz uma segurança do trabalho, para evitar explosões de reatores ou alguma elevação brusca de temperatura. Então, a gente está trazendo uma melhoria nas condições de trabalho, além das condições ambientais e menos gastos com os processos de tratamento dos efluentes.

O conselho que eu dou para os alunos é que estudem bastante, não só para ver os novos e gerar novos processos, mas também para ver os processos que já existem e verificar de que forma eles podem tornar esses processos mais sustentáveis e trazer mais fontes renováveis. Eu disse na minha tese de doutorado que, quando a gente traz esse desenvolvimento — principalmente na área dos biocombustíveis —, tornar o nosso país, se não totalmente independente, mas com uma menor dependência dos combustíveis fósseis, faz com que a gente evita até possíveis guerras no futuro pelas fontes de petróleo. Então, eu acho que quem decide pesquisar essa área está acompanhando o desenvolvimento sustentável e ajudando o progresso do país. Esse é o meu conselho: estudem bastante para desenvolver os novos processos.

Entrevistador: Verdade. Professora, em nome de toda a equipe da conferência, eu gostaria de agradecer a senhora pela disponibilidade de nos receber esta tarde e nos conceder essa entrevista. Espero ter a oportunidade de encontrar a senhora em novembro.

Dra. Carvalho: Olha, Luiz, eu que agradeço a oportunidade de falar um pouco do meu trabalho. Nem eu sabia tanto de mim quanto as suas perguntas! Fiquei muito feliz, viu? Muito

obrigada e espero encontrar você e todas as pessoas que vão participar em novembro também.

Entrevistador: Muito obrigado.

Esta entrevista fez parte do projeto de parceria de divulgação científica interinstitucional da conferencia SSCON – 20024, segue para a futura edição em 2026 RJ.

Imagem: Logotipo da SSCON 2026.

DECLARAÇÕES

1. Limitações: A entrevista limita-se ao seu conteúdo.

2. Fonte d financiamento: O anfitrião financiou esta entrevista.

3. Conflitos de interesses: O anfitrião trabalha para a revista há muitos anos e isso pode ter influenciado a entrevista.

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Para saber mais:

https://www.researchgate.net/profile/Ana-Carvalho-65

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Se você gostou desta entrevista, talvez estas outras entrevistas possam ser do seu interesse:

- (2025). ENTREVISTA COM O VICE-REITOR DR. O. A. OMOTESHO, UNIVERSIDADE DE ILORIN, NIGÉRIA. *Periodico Tche Quimica*, *22*(49), 7. https://doi.org/10.52571/PTQ.v22.n49.2025_08_OMOTEJO_pgs_93_99.pdf
- (2025). DA FORÇA AÉREA AO FUTURO HIPERSÔNICO: A TRAJETÓRIA DE ÉLCIO GERÔNIMO DE OLIVEIRA NO DESENVOLVIMENTO AEROESPACIAL BRASILEIRO. *Periodico Tche Quimica*, *22*(49), 8. https://doi.org/10.52571/PTQ.v22.n49.2025_04_OLIVEIRA_pgs_45_52.pdf
- (2024). DA ACADEMIA À INDÚSTRIA: UMA TRAJETÓRIA DE INOVAÇÃO EM ENGENHARIA QUÍMICA - ENTREVISTA COM O PROFESSOR FERNANDO LUIZ PELLEGRINI PESSOA. *Periodico Tche Quimica*, *21*(48), 7. https://doi.org/10.52571/PTQ.v21.n48.2024_08_PELLEGRINI_pgs_109_115.pdf
- (2024). EMPREENDEDORISMO, INOVAÇÃO E ENSINO POR COMPETÊNCIAS - ENTREVISTA COM PROF. PAULO CÂMARA. *Periodico Tche Quimica*, *21*(48), 8. https://doi.org/10.52571/PTQ.v21.n48.2024_05_PAULO_pgs_73_80.pdf
- (2024). EXPLORANDO A CARREIRA E CONTRIBUIÇÕES DE JOSÉ RIBAMAR: UMA ENTREVISTA COM O PRESIDENTE DO CFQ. *Periodico Tche Quimica*, *21*(47), 16. https://doi.org/10.52571/PTQ.v21.n47.2024_06_Ribamar_pgs_69_85.pdf
- (2024). DESVENDANDO AS COMPLEXIDADES DA MEDICINA CARDIOVASCULAR: UMA CONVERSA COM O DR. PETER MCCULLOUGH (VERSÃO EM PT-BR). *Periodico Tche Quimica*, *21*(46), 8. https://doi.org/10.52571/PTQ.v21.n46.2024_05_McCullough_pgs_26_33.pdf
- (2024). O PAPEL DA DIETA MEDITERRÂNEA NA PREVENÇÃO DA INFERTILIDADE MASCULINA: UMA ENTREVISTA COM O DR. MIGUEL FORNES (VERSÃO EM PT-BR). *Periodico Tche Quimica*, *21*(46), 8. https://doi.org/10.52571/PTQ.v21.n46.2024_03_Fornes_pgs_11_18.pdf
- (2016). ENTREVISTA COM MARKUS KAYSER. *Periodico Tche Quimica*, *13*(26), 5. https://doi.org/10.52571/PTQ.v13.n26.2016.9_Periodico26_pgs_9_13.pdf

- (2009). ENTREVISTA – Dr. BILL COSTA, TECPAR – PR. *Periodico Tche Quimica*, *6*(12), 6. https://doi.org/10.52571/PTQ.v6.n12.2009.34_Periodico12_pgs_33_38.pdf

- (2007). ENTREVISTA COM ANA CLAUDIA STURARO. *Periodico Tche Quimica*, *4*(07), 2. https://doi.org/10.52571/PTQ.v4.n07.2007.JANEIRO/4_pgs_31_32.pdf

- (2005). ENTREVISTA COM PROFESSOR BENOIT B. MANDELBROT. *Periodico Tche Quimica*, *2*(03), 4. https://doi.org/10.52571/PTQ.v2.n03.2005.JANEIRO/2_pgs_13_16.pdf

- (2004). Um homem que vale a pena conhecer, Pierre Lehmann. *Periodico Tche Quimica*, *1*(02), 2. https://doi.org/10.52571/PTQ.v1.n02.2004.AGOSTO/2_pgs_10_11.pdf

- (2004). Entrevista com o Professor Gustavo Paia. *Periodico Tche Quimica*, *1* (01), 3. https://doi.org/10.52571/PTQ.v1.n01.2004.JANEIRO/3_pgs_14_16.pdf

- (2004). ENTREVISTA COM JERSON KELMAN. *Periodico Tche Quimica*, *1*(02), 4. https://doi.org/10.52571/PTQ.v1.n02.2004.AGOSTO/1_pgs_6_10.pdf

- (2004). ENTREVISTA PROFESSOR PH.D. SEBASTIÃO J. FORMOSINHO. *Periodico Tche Quimica*, *1*(01), 5. https://doi.org/10.52571/PTQ.v1.n01.2004.JANEIRO/2_pgs_9_13.pdf

- (2004). ENTREVISTA COM PROFESSOR PH.D. LAVINEL G. IONESCU. *Periodico Tche Quimica*, *1*(01), 3. https://doi.org/10.52571/PTQ.v1.n01.2004.JANEIRO/1_pgs_6_8.pdf

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

DA GOIABEIRA AO LABORATÓRIO: UMA SEQUÊNCIA DIDÁTICA EM QUÍMICA ANALÍTICA

FROM GUAVA TREE TO LABORATORY: A DIDACTIC SEQUENCE IN ANALYTICAL CHEMISTRY

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RESUMO

Introdução: O ensino de química analítica no nível superior enfrenta o desafio de integrar teoria e prática para desenvolver o senso crítico e científico dos alunos. Metodologias que contextualizam o conhecimento, como sequências didáticas com experimentos práticos e replicáveis, são essenciais para preparar estudantes para o mercado de trabalho. **Objetivo:** Relatar uma sequência didática de três ensaios práticos, de baixo custo, para o ensino de química analítica a estudantes de Ciências Biológicas. O estudo visa familiarizar os alunos com as técnicas de cromatografia e espectrofotometria, usando folhas de goiabeira como material-base. **Métodos:** O estudo seguiu uma sequência didática com três atividades. Amostras de folhas de goiabeira foram submetidas à cromatografia clássica em coluna e em camada delgada (CCD) para separar e visualizar pigmentos. A detecção de flavonoides foi realizada com cloreto de alumínio. Em seguida, um extrato metanólico foi preparado e sua capacidade antioxidante determinada por espectrofotometria, usando o teste de DPPH e calculando o valor de EC_{50}. **Resultados e Discussão:** A cromatografia em coluna separou eficientemente pigmentos como carotenóides e clorofilas. A CCD confirmou a separação e indicou a possível presença de flavonoides, reforçando o perfil fitoquímico da planta. O teste antioxidante demonstrou um valor de EC_{50} de 15,42 µg/mL para o extrato, evidenciando sua atividade biológica. A metodologia de ensino provou ser eficaz para integrar teoria e prática, promovendo o engajamento e a compreensão das técnicas. **Conclusões:** A sequência didática é uma ferramenta valiosa para o ensino de química analítica, pois promove o aprendizado técnico e o pensamento crítico. Os resultados validaram a metodologia como um modelo replicável para a educação em ciências. Futuras pesquisas poderiam aprofundar a identificação dos compostos com equipamentos mais sofisticados

Palavras-chave: *Educação, Cromatografia, Espectrofotometria, Antioxidante, Ensino*

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ABSTRACT

Background: Teaching analytical chemistry at the university level faces the challenge of integrating theory and practice to develop students' critical and scientific thinking. Methodologies that contextualize knowledge, such as practical, replicable didactic sequences, are essential for preparing students for the job market. **Aim:** To report a low-cost, practical didactic sequence of three experiments for teaching analytical chemistry to Biological Sciences students. The study aims to familiarize students with chromatography and spectrophotometry techniques using guava leaves as the base material. **Methods:** The study followed a three-activity didactic sequence. Guava leaf samples were subjected to classical column and thin-layer chromatography (TLC) to separate and visualize pigments. Flavonoid detection was performed using 5% aluminum chloride in methanol. A methanolic extract was prepared, and its antioxidant capacity was determined by spectrophotometry using the DPPH radical scavenging assay to calculate the EC_{50} value. **Results and Discussion**: Column chromatography efficiently separated pigments like carotenoids and chlorophylls. TLC confirmed the separation and indicated the possible presence of flavonoids, reinforcing the plant's phytochemical profile. The antioxidant test showed an EC_{50} value of 15.42 µg/mL for the extract, demonstrating its biological activity. The teaching methodology proved effective in integrating theory and practice, promoting engagement and understanding of analytical techniques. **Conclusions:** The presented didactic sequence is a valuable tool for teaching analytical chemistry, promoting students' technical learning and critical thinking. The results validated the methodology as a replicable model for science education. Future research could further refine compound identification using more sophisticated equipment.

Keywords: *Education, Chromatography, Spectrophotometry, Antioxidant, Teaching*

1. INTRODUÇÃO:

A aprendizagem é um processo contínuo de construção de conhecimento, onde cada etapa leva à aquisição de novos saberes (Martins et al., 2018). No ensino superior, especialmente em cursos de ciências exatas, biológicas e da saúde, a experiência em laboratório é fundamental. A curiosidade dos alunos por essas práticas é essencial para o desenvolvimento de um senso crítico, analítico e científico. Para atender a essa demanda, diversas metodologias de ensino têm sido utilizadas ao longo dos anos, desde as abordagens tradicionais até as mais inovadoras, como as metodologias ativas, o uso de tecnologias da informação e a promoção da autonomia total do aluno (Arruda e Siqueira, 2021).

Em vista disso, encontrar a melhor abordagem pedagógica para cada turma é crucial para o processo de ensino-aprendizagem. Uma ferramenta eficaz para esse fim são as sequências didáticas: um conjunto de atividades planejadas em etapas que guiam os alunos até o objetivo do aprendizado (Franco, 2018). Essa metodologia já se mostrou consolidada, apresentando resultados relevantes ao combinar, de forma flexível, metodologias ativas e tradicionais em todos os níveis de ensino.

A química no ensino superior se distingue da do ensino básico pela sua maior complexidade, exigindo conhecimento prévio e habilidades específicas para a realização de experimentos (Silva et al., 2020). Nesse contexto, a química analítica ganha destaque, principalmente em cursos da área biológica, pois seus métodos são fundamentais para obter informações detalhadas sobre amostras, processos e reações químicas (Dutra et al., 2022). Dentre as técnicas mais empregadas, a cromatografia e a espectrofotometria são amplamente usadas para detectar e quantificar analitos.

A cromatografia é uma técnica que separa substâncias com base na polaridade, utilizando uma fase móvel (solvente) e uma fase estacionária (adsorvente) (Collins, 2006). Existem diversos tipos, como a cromatografia em papel, em camada delgada, e a de coluna, sendo a cromatografia líquida de alta eficiência (HPLC) a mais sofisticada, sensível e seletiva, porém, de maior custo (Marston, 2007). Já a espectrofotometria baseia-se na absorção de luz por substâncias químicas, permitindo a detecção em comprimentos de onda específicos (λmax e λmin) e a quantificação de compostos, como na análise de compostos fenólicos (Dadi et al, 2022).

A goiabeira (*Psidium guajava* L.) apresenta um perfil fitoquímico caracterizado por altos teores de compostos fenólicos, taninos, ácidos fenólicos e terpenos, os quais conferem à espécie reconhecidas atividades antioxidantes e antimicrobianas (Huynh et al., 2025). Essas

propriedades tornam a planta uma candidata adequada para experimentos didáticos em química analítica, especialmente em práticas envolvendo preparo de amostras, extração e quantificação de compostos bioativos. Nessa perspectiva, o uso de materiais botânicos no ensino reforça o caráter interdisciplinar da química, permitindo a integração entre conhecimentos de métodos analíticos, química orgânica e biologia, ao mesmo tempo em que estimula o pensamento crítico por meio de atividades práticas vinculadas ao cotidiano dos alunos (Lima et al., 2022; Visentainer et al., 2011; Anjos & Miranda, 2023). No entanto, por ser ensino superior, é crucial que os alunos aprendam a usar os materiais e equipamentos de forma correta, preparando-os para o futuro no mercado de trabalho (Dutra et al., 2022).

Com o objetivo de oferecer uma experiência analítica prática a alunos de Ciências Biológicas da Universidade Estadual de Montes Claros, este estudo descreve uma sequência de três ensaios experimentais e de fácil replicação. Para isso, foram utilizadas folhas de goiaba (*Psidium guajava*) como material-base, devido à sua acessibilidade e familiaridade com o ambiente do campus universitário.

2. DESENVOLVIMENTO

A sequência didática consiste em três atividades práticas passo a passo. Durante a execução, foram realizadas abordagens comunicativas para reforçar e consolidar os conceitos básicos.

2.1. Materiais

2.1.1 Químicos

Sílica gel 60 intervalo de 0,06-0,2 mm 70-230 mesh (Neon, Brasil); hexano P.A, acetato de etila P.A, acetona P.A (Hexis, Brasil); ácido acético glacial P.A, ácido fórmico P.A (Synth, Brasil); metanol P.A (Neon, Brasil), Cloreto de alumínio P.A (Dinâmica, Brasil); DPPH (2,2-difenil-1-picrilhidrazil) (Sigma-Aldrich, USA); placas de alumínio pré-revestidas com sílica gel 60, de 0,2 mm de espessura, com um indicador fluorescente F 254 (Macherey-Nagel, Alemanha).

2.1.2 Vidrarias e equipamentos

Bureta de 50 mL; capela de fluxo laminar com luz UV-254nm; luz negra UV-365-395nm (Luatek, Brasil); gral e pistilo (Chiarotti, Brasil); béqueres; tubos de ensaio; pipetas Pasteur; frasco de vidro com 12cm de diâmetro; frasco de vidro com capacidade de 1L; frasco com borrifador spray; Micropipetas monocanal de 10-100, 20-200 e 100-1000 uL (Eppendorf, Alemanha); ponteira para micropipeta de 0,5-10 uL (Kasvi, Brasil); papel filtro (Brigitta, Brasil); evaporador rotativo (SP labor, Brasil); triturador IKA A11 (IKA, Brasil); espectrofotômetro UV-VIS 2550 (Shimadzu, Kyoto).

2.1.3 Obtenção da amostra vegetal

Como parte da atividade prática, foi coletado 90 gramas de folhas adultas, saudáveis e sem lesões ou marcas de herbivoria de um exemplar de goiabeira (*Psidium guajava*). A coleta se deu dentro do campus da universidade, às 13h e 15 minutos do dia 13 de maio de 2025, com as coordenadas 16W 43' 15" , 43S 52' 37". A identificação da espécie vegetal foi realizada pelo responsável pela aula prática, com base nas características taxonômicas e estruturais da espécie. Por se tratar de uma atividade educacional, sem fins de pesquisa posterior, o material não foi depositado em herbário. Parte do material foi utilizado fresco para as aulas e outra parte do material foi submetido à secagem em estufa de circulação de ar forçada por sete dias a temperatura 40º. Foi avaliado como material seco o peso constante da amostra durante a secagem em estufa.

2.2. Métodos

2.2.1 Preparo da coluna cromatográfica

Uma suspensão foi preparada com 30 gramas de sílica e 50 mL de uma mistura de hexano:acetona:acetato de etila (60:40:5, v/v/v), fase móvel com bons resultados na separação de pigmentos vegetais (Veloso et al., 2025). Para o empacotamento da coluna, um pedaço de algodão foi colocado na parte inferior para conter a fase estacionária. Após a sílica ser compactada, adicionou-se uma alíquota de 35 mL da mesma mistura e o fluxo foi verificado em mL por minuto.

2.2.2 Preparo do extrato de folhas frescas

Aproximadamente 5g de folhas frescas de goiabeira foram trituradas em um gral. Em seguida, foram adicionados 5 mL de uma solução de hexano:acetona:acetato de etila (na proporção 60:40:5, v/v/v) e o material foi macerado com um pistilo para extrair os compostos orgânicos (Veloso et al., 2025). O extrato foi então coletado, filtrado e uma alíquota de 0,5 mL foi aplicada na coluna cromatográfica para a eluição.

2.2.3 Sistema de eluição

Duas fases móveis foram usadas para a eluição. A primeira era uma mistura de hexano:acetona:acetato de etila (60:40:5, v/v/v), e a segunda, uma mistura de acetato de etila:ácido

acético glacial:ácido fórmico:água (100:11:11:26, v/v/v/v) (Wagner e Bladt, 1996). O segundo sistema foi introduzido após a eluição das substâncias de menor polaridade.

2.2.4 Coleta e agrupamento das frações

Durante a eluição, frações de 2 mL foram coletadas e caracterizadas visualmente pela cor. A similaridade química dessas frações foi posteriormente avaliada por cromatografia em camada delgada.

2.2.5 Cromatografia em camada delgada

As frações agrupadas foram analisadas por cromatografia em camada delgada (CCD), usando o mesmo solvente da coluna: hexano:acetona:acetato de etila (60:40:5, v/v/v). A análise foi feita em uma cuba saturada, e os resultados observados sob luz visível, luz UV em 254 nm e 365-395 nm. Em seguida, um revelador químico de cloreto de alumínio a 5% em metanol foi aplicado para observação sob luz UV (365-395 nm), de acordo com Sultana et al (2024) com modificação de 2% para 5%, com ganho no processo de detecção. Os dados foram então processados e alinhados no CorelDraw.

2.2.6 Preparo do extrato metanólico

Folhas de goiabeira (30g), coletadas e secas previamente, foram submetidas a uma maceração exaustiva por sete dias, com agitação ocasional. Para obter o extrato hidrometanólico, utilizou-se a proporção de 1g de material vegetal em pó para 10 mL de solvente, de acordo com Manikandan et al. (2013), com adaptação de 1:6 para 1:10, com maiores teores de solvente possibilitando melhor extração e maior superfície de contato entre o pó vegetal e o solvente. Após o período de extração, o material foi filtrado, seco e teve seu rendimento calculado.

2.2.7 Avaliação da capacidade antioxidante

A capacidade antioxidante foi determinada por meio do método espectrofotométrico de sequestro de radicais livres com DPPH (Brand-Williams; Cuvelier; Berset, 1995). As concentrações do extrato bruto e das frações (2,0 mL) variaram de 2,0 a 95,0 $\mu g.mL^{-1}$ e foram misturadas a 3,0 mL de uma solução etanólica de DPPH de 40,0 $\mu g.mL^{-1}$. Após agitação, as misturas foram incubadas no escuro por 30 minutos, e a absorbância foi medida a 517 nm em um espectrofotômetro Shimadzu UV-Vis. O ácido gálico foi utilizado como controle positivo (0,8 a 2,4 $\mu g.mL^{-1}$), e a absorbância da solução de DPPH foi medida como controle negativo.

A capacidade antioxidante foi calculada usando uma equação linear para determinar o valor de EC_{50} — a concentração necessária para inibir 50% dos radicais DPPH (Mbaebie; Edeoga; Afolayan, 2012). O Índice de Atividade Antioxidante (AAI) foi calculado pela razão entre a EC_{50} e a concentração do DPPH, sendo considerado forte quando o valor de AAI é superior a 2 (Scherer; Godoy, 2009). Todos os ensaios foram realizados em triplicata. Com os valores de absorbância, a porcentagem de atividade antioxidante foi calculada pela equação (Rufino et al., 2007):

{(AbsCont—AbsAmos)/AbsCont} × 100

onde:

AbsCont: representa o valor de absorbância do controle;

AbsAmos: representa o valor de absorbância da amostra.

3. RESULTADOS E DISCUSSÃO:

3.1. Resultados

3.1.1 Análises cromatográficas

A coluna foi empacotada de maneira que a fase estacionária ficasse distribuída de forma homogênea. O fluxo de eluição foi de 2 mL por minuto. Após a aplicação do extrato de folhas de goiabeira, os pigmentos foram carreados pelo solvente, formando faixas verdes e amarelas ao longo da coluna (Figura 1).

Figura 1. *Processo de eluição dos pigmentos de*

goiabeira (Psidium guajava). Fonte: Autores (2025)

Foram coletadas 39 frações (Figura 2) durante o processo de eluição. Quando aplicada a fase móvel 1, observou-se que a fração 1 era incolor, as frações 2 a 4 apresentaram coloração amarela (indicando presença de carotenoides) e a fração 5 apresentou coloração amarelo-esverdeada, sugerindo uma transição entre carotenóides e clorofilas. As frações 6 a 8 mostraram coloração verde azulada, possível clorofila α, enquanto as frações 9 a 11 indicaram coloração esverdeada, compatível com clorofila β. As frações 12 a 24 exibiram tons amarelos mais claros, sendo possivelmente compostas por xantofilas. As frações 25 a 32 foram incolores, enquanto as frações 33 a 37 apresentaram coloração amarela. As frações 38 e 39 apresentaram coloração levemente esverdeada.

A separação cromatográfica dos pigmentos extraídos demonstrou a eficiência da técnica. A fase móvel 1, composta por hexano, acetona e acetato de etila, facilitou a eluição de substâncias menos polares, como os carotenóides, enquanto a adição de solventes mais polares, como acetona e acetato de etila, permitiram a migração de substâncias como clorofilas e xantofilas. Quando foi adicionado a fase móvel 2, houve um aumento da polaridade, o que ajudou na eluição de outras substâncias mais polares.

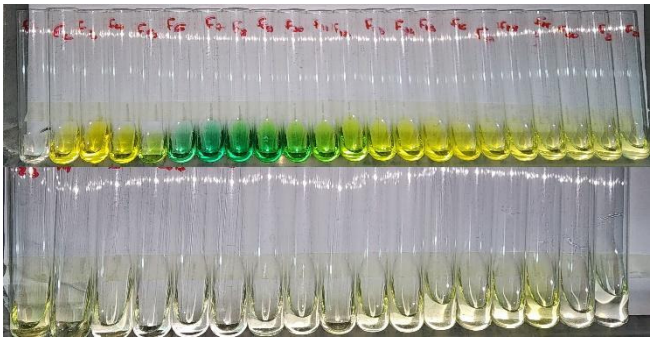

Figura 2. *Frações coletadas do extrato de goiabeira. Fonte: Autores (2025).*

As frações coletadas foram analisadas e agrupadas de acordo com a classe de pigmentos predominantes, sendo organizadas em: Frações Carotenóides (F1 e 2), Frações Clorofilas (F3-10), Frações Xantofilas (F11-34), Frações Polares (F35-39) e aplicados 30 µL de cada amostra com o auxílio de capilares, e a amostra do extrato bruto como referência (Figura 3-6).

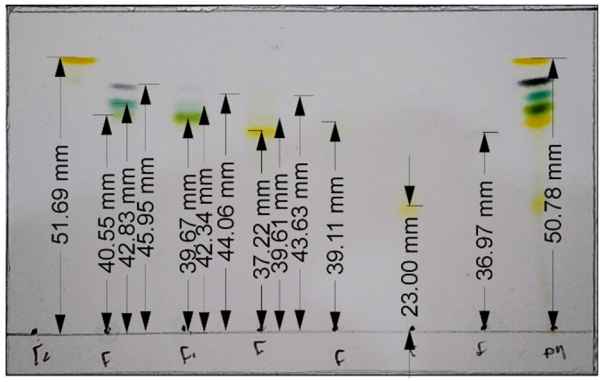

Figura 3. *A- Luz visível. Fonte: Autores (2025).*

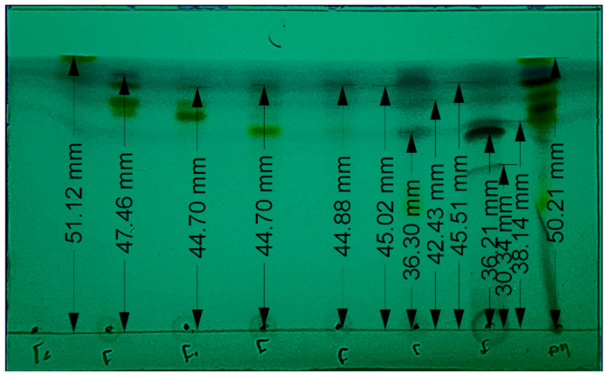

Figura 4. *Luz UV-254nm. Fonte: Autores (2025).*

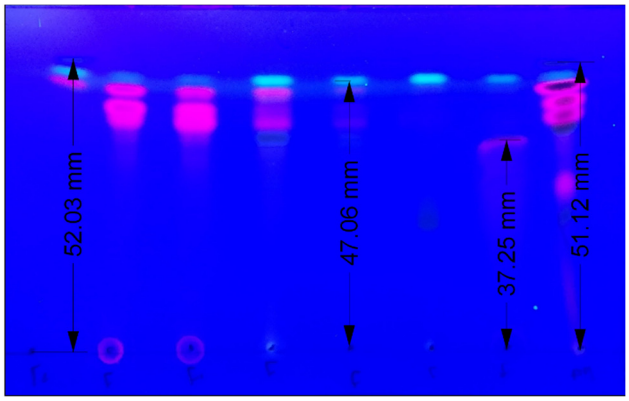

Figura 5. *Luz UV-365-395nm. Fonte: Autores (2025).*

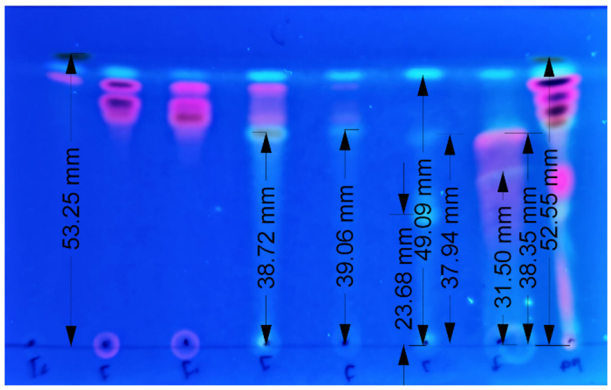

Figura 6. *Após revelação com cloreto de alumínio 5%. Fonte: Autores (2025).*

Sob luz visível, a fração FC1 apresentou coloração compatível com carotenoides (Rf ≈ 1,0). A FCL1 revelou compostos com Rf 0,89, 0,83 e 0,77, compatíveis com feofitina, clorofila A e B. A FCL2 mostrou predominância de clorofila B (Rf = 0,77). As frações FX1, FX2 e FX3 indicaram possíveis xantofilas (Rf = 0,72 e 0,44). A FP1 teve coloração levemente amarelada (Rf = 0,72). Sob luz UV 254 nm, foram observadas bandas fluorescentes em todas as frações, com destaque para Rf = 0,90 e bandas adicionais em F34 e F39 (Rf = 0,72). Em 365 nm, todas revelaram uma substância fluorescente comum (Rf = 0,91) e F39 mostrou uma segunda fluorescência (Rf = 0,72). Após uso do revelador de cloreto de alumínio, foi observada luminescência em todas as frações, com destaque para substâncias adicionais nas frações FX1, FX2, FX3 e FP1.

3.1.2 Rendimento do extrato hidrometanólico

O extrato hidrometanólico seco foi pesado e feito o cálculo de rendimento, no qual obteve-se 30% de rendimento total, ou 300 mg. O extrato obtido possui coloração marrom, com brilho característico de extratos vegetais.

3.1.3 Capacidade antioxidante

Na determinação da capacidade antioxidante, foi utilizado o teste *in vitro* com 2,2-difenil-1-picrilhidrazila (DPPH). Para determinar a EC_{50} em ug/mL, foi utilizada a equação da reta padrão para o ácido gálico e para a amostra (Gráfico 1) (Tabela 1). O R^2 para o extrato metanólico de folha de goiabeira foi de 0.9796 (EC_{50} = 15,43 ± 1,49 ug/mL) (Tabela 1)(Gráfico 2). O índice da atividade antioxidante foi calculado para o ácido gálico, com o valor de 13,29, e para o extrato que foi de 2,59.

Tabela 1. *Dados ensaio DPPH ácido gálico e goiabeira*

Amostra	EC50 ± DP* ug/mL
Ácido gálico	3,01 ± 0,01
EXMFG**	15,43 ± 1,49

**Desvio padrão, ** Extrato hidrometanólico de folhas de goiabeira. Fonte: Autores (2025).*

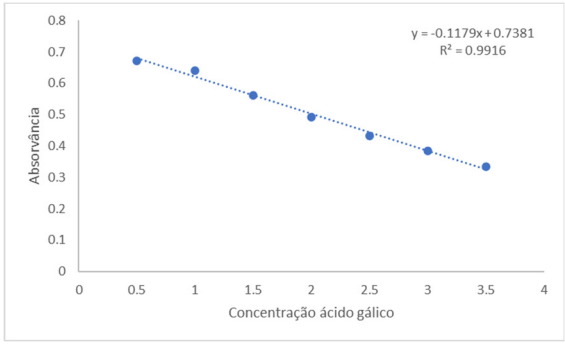

Gráfico 1 - *Curva padrão ácido gálico. Fonte: Autores (2025)*

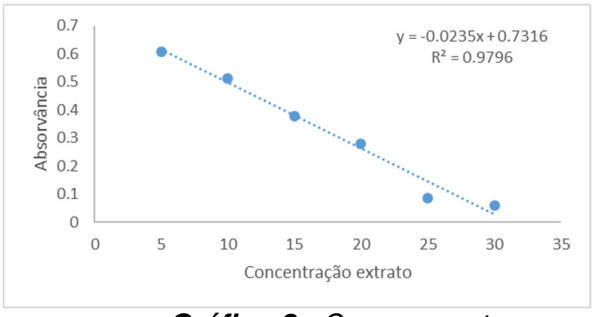

Gráfico 2 *- Curva amostra. Fonte: Autores (2025)*

3.2. Discussão

Ao promover atividades práticas em laboratório, constrói-se uma importante ponte do conhecimento, não de forma bancária, mas tecnicista, abordando métodos e técnicas que possam ser aplicadas no futuro (Arruda e Siqueira, 2021). Especificamente no presente trabalho, a construção do conhecimento por meio de um processo sequencial favorece o aprendizado em etapas. Logo a assimilação das atividades e contexto ganham sentido e promovem a interação entre os envolvidos. Essas ações, do aprender e fazer, encaixam-se no pensamento freiriano da autonomia, uma vez que ensinar exige reconhecer os envolvidos como sujeitos de sua própria aprendizagem, e a partir disso construir o conhecimento junto por meio de diálogos (Freire, 1996).

A atividade inicial consistiu na preparação e eluição de pigmentos vegetais por cromatografia clássica em coluna, na qual a escolha dos solventes de extração, assim como os sistemas de eluição promoveram a separação dos pigmentos pela polaridade (Collins et al., 2006, Veloso et al., 2024, Veloso et al., 2025). A funcionalização final com outro sistema de solvente, esse já aplicado em cromatografias planares para a eluição de flavonoides, foi utilizada como estratégia para retirada de resíduos que poderiam estar aderidos à sílica.

A eluição inicial promoveu a separação de grupos de pigmentos, o primeiro de carotenos/carotenóides (amarelo), o segundos das clorofilas A (verde azulado) e B (verde amarelado) e o terceiro das xantofilas (amarelo e tons amarelados), o mesmo pode ser observado em trabalho que envolve a cromatografia planar (Veloso et al., 2025). Conjunto de pigmentos já reconhecidos pela afinidade com a fase móvel a qual foi utilizada na coluna (Collins et al., 2006). A segunda eluição, com o maior índice de polaridade relativa, além de promover a limpeza da coluna, correu substâncias residuais, possivelmente flavonoides.

A cromatografia em camada delgada, realizada posteriormente à coluna clássica possibilitou avaliar a separação e agrupamento das frações, assim como averiguar a pureza do que foi eluído (Marston, 2007). As frações que continham carotenos e xantofilas apresentaram maior teor de separação e isolamento quando comparado às clorofilas, que apesar de aparentemente bem isoladas, a similaridade não proporcionou a separação máxima. Além da observação em luz visível a utilização de luz UV-254nm e 365-395nm possibilitou a observação de pontos com demarcações não observáveis anteriormente (245 nm) e fluorescência (365-395 nm) antes e após a revelação da placa com cloreto de alumínio a 5%. A fluorescência intensificada após a utilização do revelador, pode indicar a presença de flavonoides na amostra, o que corrobora com o próprio perfil químico das folhas de goiabeira, que possuem flavonoides aglicona e glicosilados em suas folhas (Marston, 2007).

O extrato produzido anteriormente, foi preparado na concentração de 30ug/mL, e triagem inicial realizada na busca da faixa ideal para o ensaio da capacidade antioxidante. Esse realizado sob observação e orientação, no qual foi determinado o EC_{50} da amostra, ou seja a capacidade do extrato de neutralizar 50% dos radicais livres. O resultado encontrado para o padrão positivo, o ácido gálico foi de 3,01 ± 0,01 ug/mL e para o extrato foi de 15,43 ± 1,49 ug/mL. Excelente resultado quando comparado ao ácido gálico, substância pura, conhecida pela alta capacidade antioxidante.

Além dos resultados analíticos obtidos, a aplicação desta sequência prática em contexto didático favorece a construção do conhecimento, uma vez que os estudantes serão desafiados a interpretar reações químicas, propriedades estruturais dos metabólitos com seus comportamentos cromatográficos e compreender a lógica dos cálculos envolvidos na determinação do EC_{50}, aliando duas características, ilustrativa e investigativa (Oliveira, Barbosa e Flores, 2020). Além disso, a execução dessa sequência exige infraestrutura mínima de laboratório (placas de CCD, solventes, lâmpadas UV), o que pode limitar a replicação em instituições com recursos reduzidos, sendo considerada uma barreira amplamente relatada em práticas de química analítica no ensino superior (Apkarian et al., 2021).

O experimento levou a capacidade do pensamento, e a indução de questões relacionadas ao funcionamento dos reagentes e da amostra, assim como a realização dos cálculos (Dadi et al., 2022).

4. CONCLUSÕES:

Este estudo demonstrou a eficácia de uma sequência didática para o ensino de química analítica a estudantes de Ciências Biológicas. O trabalho utilizou a cromatografia e a espectrofotometria para a separação e caracterização de compostos em folhas de goiabeira, uma amostra acessível e relevante para os alunos.

As principais contribuições da pesquisa incluem a integração de conceitos teóricos e práticos por meio de experimentos de baixo custo e replicáveis. Isso não apenas enriquece o processo de aprendizagem, mas também desenvolve o senso crítico e científico dos alunos, preparando-os para futuros desafios na área.

O trabalho concluiu que a sequência didática é uma ferramenta de ensino valiosa, que promove o engajamento e a compreensão das técnicas analíticas. Os resultados obtidos confirmaram a presença de diferentes pigmentos e a capacidade antioxidante da amostra, validando a metodologia e alcançando o objetivo principal do estudo. Futuros trabalhos poderiam aprofundar a identificação dos compostos usando técnicas mais avançadas, mas a base pedagógica aqui estabelecida já se mostra um importante avanço.

5. DECLARAÇÕES

5.1. Limitações do Estudo

Este estudo apresenta algumas limitações que merecem ser destacadas. A principal delas reside na ausência de análises mais aprofundadas para a identificação e quantificação precisa dos compostos isolados. Para tal, seria necessário o uso de equipamentos mais

avançados, como a cromatografia líquida de alta eficiência (HPLC) acoplada à espectrometria de massas (LC-MS), que não estavam disponíveis. A análise se baseou principalmente em métodos de baixo custo e facilmente replicáveis, o que, embora tenha atendido ao propósito didático do trabalho, restringe a profundidade da caracterização química dos extratos. Além disso, a avaliação da capacidade antioxidante, realizada in vitro com o método DPPH, não reflete necessariamente a atividade biológica in vivo.

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5.4. Conflitos de Interesse

Os autores declaram não haver conflitos de interesse nem interesses concorrentes.

5.5. Disponibilidade de Dados

Option A: Data Available in Manuscript (Opção A: Dados Disponíveis no Manuscrito) ‘’All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author’’

5.6. Contribuições dos Autores

Luccas Roberto Pereira Mendes: CD, DC, DAI, MW, CR; Pedro Henrique Fonseca Veloso: CD, DC, DAI, MW, CR, FA; Christian de Almeida Soares: MW; Ellen Laureany Araujo Olimpo and Eurislene Moreira Antunes Damasceno: DAI; Vanessa de Andrade Royo: CR.

5.7. Declaração de Ferramentas de IA e Computacionais

Option A: No AI Tools Used

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Declaração de Integridade da Pesquisa

- No data fabrication
- No results falsification
- No P-hacking or selective reporting
- Original work
- Not previously published
- Methods conducted ethically

5.9. Declaração de Originalidade e Plágio

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5.10. Licença de Acesso Aberto

6. ESTUDOS RELACIONADOS COM HUMANOS E ANIMAIS

6.1. Aprovação do Comitê de Ética

Não aplicável.

6.2. Consentimento Informado

Não aplicável.

7. REFERÊNCIAS:

1. Anjos, F. B., & Miranda, A. C. G. (2023). Sequência didática fundamentada em experimentação: Uma estratégia para o ensino de tabela periódica e reações químicas a partir de materiais alternativos de baixo custo. *Research, Society and Development*, 12(8). https://doi.org/10.33448/rsd-v12i8.43085

2. Apkarian, N., *et al.* (2021). What really impacts the use of active learning in undergraduate STEM education? Results from a national survey of chemistry, mathematics, and physics instructors. *PLOS ONE*, **16**(2), e0247544. https://doi.org/10.1371/journal.pone.0247544

3. Arruda, J. S., & Siqueira, L. M. R. de C. (2020). Metodologias ativas, ensino híbrido e os artefatos digitais: Sala de aula em tempos de pandemia. *Práticas Educativas, Memórias e Oralidades – Revista Pemo*, **3**(1), e314292. https://doi.org/10.47149/pemo.v3i1.4292

4. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, **28**, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

5. Collins, C. H., Braga, G. L., & Bonato, P. S. (2006). *Fundamentos de cromatografia*. Campinas: Editora Unicamp.

6. Dadi, M., & Yasir, M. (2022). Spectroscopy and spectrophotometry: Principles and applications for colorimetric and related analysis. *Colorimetry*, **1**, 81–102. https://doi.org/10.5772/intechopen.101106

7. Dutra, J. W. A., Moreira, A. M. D., Pereira, A. I. S., Ribeiro, F. A. A., Rojas, M. O. A. I., Moreira, D. S., Santos, I. P., Reis, E. F., Oliveira, E. S., & Silva, J. R. (2022). Ferramentas educativas digitais para o ensino de química analítica. *RECIMA21 – Revista Científica Multidisciplinar*, **3**(2), e321101. https://doi.org/10.47820/recima21.v3i2.1101

8. Franco, D. L. (2018). A importância da sequência didática como metodologia no ensino da disciplina de física moderna no ensino médio. *Revista Triângulo*, **11**(1). https://doi.org/10.18554/rt.v0i0.2664

9. Freire, P. (1996). *Pedagogia da autonomia: Saberes necessários à prática educativa* (27ª ed.). São Paulo: Paz e Terra.

10. Huynh, H. D., *et al.* (2025). Bioactive compounds from guava leaves (*Psidium guajava* L.): Characterization, biological activity, synergistic effects, and technological applications. *Molecules*, **30**(6), 1278. https://doi.org/10.3390/molecules30061278

11. Lima, L. P., *et al.* (2022). A utilização de produtos naturais como alternativa para o ensino de química: Uma revisão. *Pesquisa, Sociedade e Desenvolvimento*, 11(7), e2111729588. https://doi.org/10.33448/rsd-v11i7.29588

12. Manikandan, R., Anand, A. V., & Muthumani, G. D. (2013). Phytochemical and in vitro anti-diabetic activity of methanolic extract of *Psidium guajava* leaves. *International Journal of Current Microbiology and Applied Sciences*, 2(2), 15–19. http://www.ijcmas.com/Archives/vol-2-2/Manikandan%20etal.pdf

13. Marston, A. (2007). Role of advances in chromatographic techniques in phytochemistry. *Phytochemistry*, **68**(22–24), 2786–2798. https://doi.org/10.1016/j.phytochem.2007.08.004

14. Martins, E. D., Moura, A. A. de, & Bernardo, A. de A. (2018). O processo de construção do conhecimento e os desafios do ensino-aprendizagem. *Revista On-line de Política e Gestão Educacional*, **22**(1), 410–423. https://doi.org/10.22633/rpge.v22.n.1.2018.10731

15. Mbaebie, B., Edeoga, H., & Afolayan, A. (2012). Phytochemical analysis and antioxidant activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*, **2**, 118–124. https://doi.org/10.1016/S2221-1691(11)60204-9

16. Oliveira, M. C. C., Barbosa, R. C. F., & Flores, D. C. (2020). Atividade antioxidante de frutas cítricas: Adaptação do método do DPPH para experimentação em sala de aula. *Química Nova na Escola*, **43**(4), 401–405. https://doi.org/10.21577/0104-8899.20160251

17. Rufino, M. D. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. D. G., Pérez-Jiménez, J., & Saura-Calixto, F. (2007). *Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH* (Comunicado Técnico nº 127). Fortaleza: Embrapa Agroindústria Tropical.

18. Scherer, R., & Godoy, H. T. (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry*, **112**, 654–658. https://doi.org/10.1016/j.foodchem.2008.06.026

19. Silva, B., Silva Neto, S., & Leite, B. (2020). Sala de aula invertida no ensino da química orgânica: Um estudo de caso. *Química Nova*. https://doi.org/10.21577/0100-4042.20170677

20. Sultana, S., Hossain, M. L., Sostaric, T., Lim, L. Y., Foster, K. J., & Locher, C. (2024). Investigating flavonoids by HPTLC analysis using aluminium chloride as derivatization reagent. *Molecules*, 29(21), 5161. https://doi.org/10.3390/molecules29215161

21. Veloso, P. H. F., de Melo Sacramento, V., & de Andrade Royo, V. (2024). Uso da casca de ovo como fase estacionária alternativa para cromatografia líquida clássica em coluna. *Revista Unimontes Científica*, 26(1), 1–16. https://doi.org/10.46551/ruc.v26n1a7

22. Veloso, P. H. F., Sacramento, V. D. M., & Royo, V. D. A. (2025). Cromatografia preparativa em camada delgada: Comparativo entre placa de sílica gel e giz. *Desafios – Revista Interdisciplinar da Universidade Federal do Tocantins*, **12**(4), 1–14. https://doi.org/10.20873/2025_jul_19444

23. Visentainer, J. V.; Souza, N. E.; Visentainer, J. E. L.; Análises Químicas Qualitativas em Alimentos: Experimentos Laboratoriais Explicativos e de Fácil Realização, utilizando Alimentos, Enzimas e Reagentes do Cotidiano, 1a ed., EDUEM: Maringá, 2011.

24. Wagner, H., & Bladt, S. (2009). *Plant drug analysis: A thin layer chromatography atlas* (2nd ed.). Berlin: Springer. https://doi.org/10.1007/978-3-642-00574-9

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

Bacillus subtilis COMO CEPA PADRÃO PARA DETECTAR RESÍDUOS DE PENICILINA G E ESTREPTOMICINA EM AMOSTRAS DE LEITE

Bacillus subtilis AS A STANDARD STRAIN TO DETECT PENICILLIN G AND STREPTOMYCIN RESIDUES IN MILK SAMPLES IN KARBALA, IRAQ

Bacillus subtilis كسلالة قياسية للكشف عن متبقيات البنسلين ج والستربتوميسين في عينات الحليب في محافظة كربلاء، العراق

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RESUMO

Introdução: O uso inadequado e não regulamentado de medicamentos veterinários na produção de alimentos de origem animal pode ser prejudicial tanto à saúde do consumidor quanto à indústria leiteira. A presença de resíduos de antibióticos no leite contribui para o surgimento de bactérias resistentes a antibióticos, que podem ser transmitidas dos animais aos seres humanos, levando ao desenvolvimento de alergias e ao desequilíbrio da microflora intestinal. **Objetivo**: Este estudo teve como objetivo detectar resíduos dos antibióticos penicilina G e estreptomicina em leite pasteurizado e leite cru na cidade de Karbala, utilizando um isolado local de Bacillus subtilis. **Métodos**: *B. subtilis* HS foi isolado de uma área agrícola e utilizado como micro-organismo teste para a detecção de resíduos de antibióticos em amostras de leite. Um total de 45 amostras de leite, incluindo 32 leites crus provenientes de diferentes fazendas e 13 leites pasteurizados adquiridos em mercados, foi coletado no período de setembro de 2024 a abril de 2025. Os resíduos de antibióticos nas amostras de leite foram detectados por meio do método de difusão em poço em ágar e cromatografia em camada delgada

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(CCD). Resultados: O ensaio de difusão em poço em ágar demonstrou que, das 45 amostras analisadas (4/13 de leite pasteurizado e 12/32 de leite cru), 30,76% e 37,5% foram positivas, respectivamente. As amostras de leite apresentaram uma taxa de prevalência total de 35,5% para resíduos de antibióticos. Os resultados da CCD evidenciaram a presença de resíduos de penicilina G em 9 amostras de leite (56,25%), sendo 1 de leite pasteurizado e 8 de leite cru, as quais apresentaram mancha idêntica à do antibiótico padrão ao valor de Rf de 0,85. Quanto à presença de resíduos de estreptomicina, 2 amostras (12,5%) de leite cru apresentaram mancha idêntica à do antibiótico padrão ao valor de Rf de 0,51. Ambos os resíduos de antibióticos foram detectados simultaneamente em 2 amostras (12,5%) de leite cru. **Discussão**: A maior ocorrência de resíduos de antibióticos no leite cru em comparação ao leite pasteurizado pode ser atribuída ao não cumprimento, por parte dos produtores, dos períodos de carência estabelecidos pela Comissão do Codex Alimentarius após a administração de medicamentos aos animais. Contribui também para esse cenário a ausência de controle sobre a comercialização do leite cru, vendido diretamente pelos produtores sem a realização de testes rigorosos. **Conclusões**: A presença de resíduos de antibióticos nas amostras de leite evidencia a necessidade de que as autoridades monitorem a qualidade do leite cru que chega ao mercado consumidor.

Palavras-chave: *Segurança alimentar, cromatografia em camada delgada, ensaio de difusão em poço em ágar, Bacillus subtilis, leite pasteurizado*.

ABSTRACT

Background: The improper and unregulated use of veterinary drugs in animal food production can be harmful to both consumer health and the dairy industry. The presence of antibiotic residues in milk contributes to the emergence of antibiotic-resistant bacteria that could be transmitted from animals to humans, leading to the development of allergies and disturbances in the balance of intestinal microflora. **Aim:** This study aimed to detect penicillin G and streptomycin antibiotic residues in pasteurized and raw milk in Karbala city using a local isolate of *Bacillus subtilis.* **Methods:** *B. subtilis* HS was isolated from an agricultural area and used as a test microorganism for antibiotic residues in milk samples. A total of 45 milk samples, including 32 raw milks from various farms and 13 pasteurized milks from markets, were collected from September 2024 to April 2025. Antibiotic residues in milk samples were detected using Agar Well diffusion method and thin-layer chromatography (TLC). **Results**. Agar well diffusion assay showed that, out of 45 samples (4/13 pasteurized milk and 12/32 raw milk), 30.76% and 37.5% were positive, respectively. The milk samples had a total prevalence rate of 35.5% for antibiotic residues. The results of TLC showed the presence of penicillin G residue was out of 9 milk samples (56.25%), 1 (pasteurized milk), and 8 (raw milk) had an identical spot with the standard antibiotic at Rf value of 0.85. Regarding the presence of streptomycin residue, 2 samples (12.5%) from raw milk showed an identical spot with the standard antibiotic at Rf of 0.51. Both antibiotic residues were detected in 2 samples (12.5%) of raw milk. **Discussion:** The higher occurrence of antibiotic residues in raw milk compared to pasteurized milk samples could be attributed to farmers not following the withdrawal periods set by the Codex Alimentarius Commission after administering medication to their animals. Also, the lack of control over raw milk sales, where farmers are sold directly without undergoing strict testing. **Conclusions:** The presence of antibiotic residues in milk samples necessitates that authorities monitor the quality of raw milk entering the consumer market.

Keywords: *Food safety, thin layer chromatography, Agar well diffusion assay, Bacillus subtills, pasteurized milk.*

الملخص

الخلفية: إنّ الاستخدام غير السليم وغير المنظَّم للأدوية البيطرية في إنتاج الغذاء الحيواني يمكن أن يُلحق أضرارًا بصحة المستهلك وصناعة الألبان على حدٍّ سواء. إذ يُسهم وجود متبقيات المضادات الحيوية في الحليب في ظهور بكتيريا مقاومة للمضادات الحيوية يمكن أن تنتقل من الحيوانات إلى الإنسان، مما يؤدي إلى حدوث حالات تحسّسية واضطرابات في توازن النبيت الجرثومي المعوي. **الهدف:** هدفت هذه الدراسة إلى الكشف عن متبقيات المضادين الحيويين البنسلين G والستربتومايسين في الحليب المبستر والحليب الخام في مدينة كربلاء باستخدام عزلة محلية من بكتيريا *Bacillus subtilis*. **طرائق العمل:** تم عزل السلالة HS *B. subtilis* من منطقة زراعية، واستُخدمت ككائن مجهري اختباري للكشف عن متبقيات المضادات الحيوية في عينات الحليب. جُمعت (45) عينة حليب، منها (32) عينة حليب خام من مزارع مختلفة و(13) عينة حليب مبستر من الأسواق، خلال المدة من أيلول 2024 إلى نيسان 2025. وتم الكشف عن متبقيات المضادات الحيوية باستخدام طريقة Agar Well diffusion وتقنية الكروماتوغرافيا ذات الطبقة الرقيقة (TLC). **النتائج:** أظهر اختبار الانتشار في الآغار بطريقة الحفر أنّه من أصل 45 عينة (13/4 من الحليب المبستر و32/12 من الحليب الخام)، كانت نسب النتائج الإيجابية 30.76% و37.5% على التوالي. وبلغت نسبة الانتشار الكلية لمتبقيات المضادات الحيوية في عينات الحليب 35.5%. وأظهرت نتائج الكروماتوغرافيا ذات الطبقة الرقيقة (TLC) وجود متبقيات البنسلين G في 9 عينات حليب (56.25%)، منها عينة واحدة من الحليب المبستر و8 عينات من الحليب الخام، حيث أظهرت بقعة مماثلة للمضاد القياسي عند قيمة Rf بلغت 0.85. أمّا بالنسبة لمتبقيات الستربتومايسين، فقد أظهرت عينتان (12.5%) من الحليب الخام بقعة مماثلة للمضاد القياسي عند قيمة Rf بلغت 0.51. كما تم الكشف عن كلا متبقيي المضادين الحيويين في عينتين (12.5%) من الحليب الخام. **المناقشة:** يُعزى الارتفاع في نسبة متبقيات المضادات الحيوية في الحليب الخام مقارنةً بالحليب المبستر إلى عدم التزام المزارعين بفترات السحب المحدّدة من قبل هيئة الدستور الغذائي الدولية بعد إعطاء الأدوية للحيوانات. كما يُسهم ضعف الرقابة على بيع الحليب الخام، حيث يُباع مباشرةً دون إخضاعه لاختبارات صارمة، في زيادة

هذه النسبة. **الاستنتاجات:** إنّ وجود متبقيات المضادات الحيوية في عينات الحليب يستدعي ضرورة قيام الجهات المختصّة بمراقبة جودة الحليب الخام الداخل إلى سوق المستهلك.

الكلمات المفتاحية: سلامة الغذاء، الكروموتوغرافيا ذات الطبقة الرقيقة، فحص الانتشار بالحفر، *Bacillus subtilis*، *الحليب المبستر*.

1. INTRODUCTION:

The mitigation of the increasing threat of antimicrobial residues in milk and dairy products is the farmer's responsibility. Antibiotics are widely used in livestock farming for disease prevention, infection treatment, and growth promotion, accounting for more than 70% of global antibiotic consumption (Allen *et al*., 2013; Jechalke *et al*., 2014; Salim *et al*., 2018). Most antibiotics used in animals are the same as or closely related to those used in humans (Marshall and Levy, 2011; Ma *et al*., 2021). It is well known that antibiotic contamination creates selective pressure for resistant bacterial strains (Tello *et al*., 2012; Arsène *et al*., 2022). The most widely used antibiotics for both animal and human are classified within the β-lactam group, which includes: carbapenem, monobactam, cephalosporin, and penicillin (Prescott and Hardefeldt, 2024; Garkavenko *et al*.,2021). Other classes include macrolides such as erythromycin and azithromycin. Aminoglycosides such as streptomycin, gentamycin (Conceição *et al*.,2023). Some antibiotics are incompletely absorbed and metabolized in animals, so the residues are excreted unchanged through urine (Chee-Sanford *et al*., 2009). Consequently, high concentrations of residual antibiotics are released in animal manure (Qian *et al*., 2018).

The presence of antibiotic residues in food products, especially milk, has certain harmful effects on public health. A study conducted by Zhang found that antibiotic residues in the muscles, kidneys, and livers of cattle and sheep, may pose a threat to human health (Zhang *et al*.,2021). Another study found antibiotic residues in milk (Titouche *et al*., 2013; Batah *et al*., 2025). Moreover, poultry may contain antibiotic residues, which can cause numerous adverse health effects in humans. Antibiotic residues have been detected in chicken production (meat and eggs) (Chang *et al*., 2015). The accumulation of penicillin residues in milk led to the development of allergies in some hypersensitive individuals, while the accumulation of sulfamethazine and oxytetracycline could increase the risk of cancer (Hou *et al*., 2015). Also, the presence of gentamicin residues is causing nephropathy and disturbances in the intestinal microbiota (Back *et al*., 2020). Therefore, the detection of veterinary drug residues in food represents an important food safety issue

The bioanalytical techniques used to detect antibiotic residues in food products are mainly categorized into two groups: screening methods and Confirmatory Methods (Cháfer-Pericás *et al*., 2010). Screening methods such as microbial inhibition tests, immunoassays like ELISA, and lateral flow tests are characterized by their quickness, ease of use, and affordability (Ghimpețeanu *et al*., 2022). They detect the presence of antibiotic residue above a certain limit rather than identifying the specific type (Cháfer-Pericás *et al*., 2010). Confirmatory methods, such as chromatography coupled with mass spectrometry (LC-MS/MS) and (GC-MS/MS), require expensive equipment and trained personnel (Wang *et al*.,2022). However, these methods are particularly powerful, capable of identifying and quantifying over 100 different antibiotics at very low levels in a single test (Berendsen *et al*.,2013).

Bacillus subtilis is referred to as a "soil dweller," with its natural niche in soil. It is a spore-forming, Gram-positive, rod-shaped, motile bacterium (Norris *& Wolf,* 1961). *B. subtilis is* considered an effective microorganism that is used to detect antibiotic residues in milk for several reasons: first, the broad sensitivity, where many studies showed that *B. subtilis* is naturally sensitive to a wide range of antibiotics such as penicillin, amoxicillin, cephalosporins, and tetracyclines (Sharma *et al*.,2025). This wide-ranging sensitivity enables a single test of *B. subtilis* to effectively detect contamination of food with a variety of different antibiotics (Lee *et al*., 2007; Navrátilová *et al*., 2024). *B. subtilis* is an ideal organism for commercial test kits because it produces spores, which are a stable, inactive state and highly resistant to both heat and drying. This characteristic gives the test kits a long shelf life and allows them to be stored and transported easily (Gondová *et al*., 2014; Trufanov *et al*., 2015). Also, microbial tests using *B. subtilis* are more practical and quicker for screening than chromatography or mass spectrometry. They are

simpler, faster, and much cheaper (Ferone *et al*.,2020).

1.1. Aims

Due to public health concerns, milk and dairy products containing antibiotic residues above safe levels are not considered safe for people to drink (Plumb, 2018). However, there's been growing concern about how often veterinary drug residues are found in food, especially milk. Despite this, no previous studies have specifically examined antibiotic residues in milk sold in Karbala Province. So, this study aims to detect these residues in pasteurized milk (market) and raw cow milk (dairy farm) in Karbala using the Agar well diffusion method and thin-layer chromatography.

2. MATERIALS AND METHODS:

2.1. Materials

2.1.1 Isolation and Identification of Bacillus subtilis

B. subtilis is isolated selectively, as recommended by Vehapi *et al*. (2023), with a slight modification. Soil samples were collected from an agricultural area in the College of science/, Karbala University. Ten soil samples, taken to a depth of 10 cm, were placed in sterile, sealed bags and transported to the laboratory. From each soil sample, 1 g was weighed and placed in a test tube containing 9 mL of sterile saline solution. To obtain a homogeneous suspension by breaking up aggregated soil particles, the samples were shaken in a vortex machine at the highest speed for 20 minutes. To obtain *B. subtilis* spores, the soil suspension from each sample was heated in a water bath at 80 °C for 20 minutes to eliminate vegetative bacterial cells. Then 10 µL of the soil suspension was transferred and spread on a solid culture surface of sterile nutrient agar. The plates were incubated at 30 °C in an incubator for 24-48 hours.

An initial confirmation test was used to identify the selected isolates, including the Gram stain and catalase tests (Amin *et al*., 2015). All isolates were identified as *B. subtilis*. After that, the Viteck 2 test was performed for more accurate identification. The strain was designated as “HS”.

2.1.2 Preparation of Bacillus subtilis HS spore suspension

The spore suspension of *B. subtilis* HS was prepared according to the method described by El Atabani *et al*. (2014). Under sterile conditions, several colonies of the bacteria were transferred to sterile nutrient agar culture plates and incubated at 30 °C for 10 days to induce bacterial cells to produce spores. At the end of the incubation period, the cells were harvested in 10 mL of sterile saline (0.8% Sodium chloride), then centrifuged at 3000 rpm for 10 minutes (repeated twice). The suspension was heated at 70 °C in a water bath for 30 minutes to kill the vegetative cells. The hot suspension was transferred to a centrifuge and spun at 3000 rpm for 10 minutes; the supernatant was discarded. Another 10 mL of sterile saline was added to wash the remnants of the vegetative cells. The mixture was concentrated at the same speed and duration (repeated twice) to obtain a pure spore’s suspension.

2.1.3 Antibiotic sensitivity test for B. subtilis HS

Antibiotic sensitivity tests for *B. subtilis* HS were performed using the Kirby-Bauer method, following the guidelines for antimicrobial disk susceptibility testing established by the Clinical and Laboratory Standards Institute (CLSI, 2012). Nine types of antibiotics (Oxoid, UK) used have been used. The selected antibiotic belonged to five classes of antimicrobials. These antibiotics include aminoglycosides: Kanamycin (K, 30 µg), Neomycin (N, 30 µg) and Streptomycin (S, 10 µg); β-lactam: Ampicillin (AM, 10 µg), Amoxicillin (AmC, 30 µg) and Penicillin G (P, 10 µg); fluroquinolones: Ciprofloxacin (CIP, 5 µg); phenicols: Chloramphenicol (C, 30 µg); tetracyclines: Tetracycline (Te, 30 µg). An overnight bacterial suspension was made to match the turbidity of a 0.5 McFarland standard. This suspension was then evenly spread across Muller Hinton agar plates using a cotton swab. Four antibiotic disks were impregnated on the plates, with a distance of 10 mm between each. The plates were transferred to a fridge for 20 minutes, then incubated at 35 °C for 24 hours. Zone inhibition around each disk was measured in millimeters (mm), and the results were recorded.

2.1.4 Milk sample collecting

Two types of milk samples were randomly collected from September 2024 to April 2025. Samples of all types were collected from Karbala

province. The first one was different brands of unflavored pasteurized milk samples (13), denoted by (P), purchased at the point of sale. The other one was raw milk samples from cows (32), denoted by (C), that were collected from different farms. The samples were kept under cold conditions until transported to the laboratory.

2.1.5 Preparation of standard antibiotic concentrations

Two antibiotics were used in this study: penicillin G and streptomycin, which were kindly supplied by the State Company for Drug Industries and Medical Appliances, Samarra, Iraq. The stock solution for each antibiotic was prepared at a concentration 0.1 mg/mL by dissolving in methanol. Working stock solutions of Penicillin G and streptomycin were sterilized using a Millipore filter and stored at 4 °C until used.

2.2. Methods

2.2.1 Detection of antibiotic residue using Agar well diffusion method

To perform this test, 100 µL of *B. subtilis* HS spore suspension (diluted with normal saline to achieve a turbidity of McFarland's standard solution (0.5) that equivalent to about $1.5x10^8$ CFU/mL) were transferred to each 100 mL of solid culture medium of Muller- Hinton agar cooled to 45 °C (prior to solidification of the medium) and mix well. The medium was then poured into sterilized Petri dishes and left to solidify at room temperature. Using a sterile cork borer, four holes (8 mm in diameter) spaced 20 mm apart were drilled into the surface of the Muller-Hinton culture medium. Each well was filled with 100 µL of the milk samples, individually. The plates were incubated at 30 °C for 24 hours under aerobic conditions. The presence of antibiotic residues was indicated by the formation of an inhibition zone around the wells (absence of bacterial growth). The inhibition zones around the wells were measured, and the results were recorded (Al-Mashhadany *et al.*,2018). The prevalence percentage of antibiotic residue is determined using Equation 1

$$Prevalence\ (\%) = \left(\frac{Number\ of\ positive\ samples}{Total\ number\ of\ samples\ tested}\right) \times 100 \quad \text{(Eq. 1)}$$

Detection of minimum inhibition zone diameter was performed by preparing a serial dilution of each antibiotic (25, 10, 5, 2, 1 µg/mL). Then add the prepared antibiotic solution to antibiotic-free milk, followed by the extraction of antibiotics from the spiked milk. The steps for the Agar well diffusion method, as mentioned above, were repeated. Then determine the lowest antibiotic concentration that yields the smallest inhibition zone (mm).

2.2.2 Detection of antibiotic residue using the thin-layer chromatography (TLC) technique

2.2.2.1 Mobile phase of TLC

The mobile phase was prepared as described by Kaya and Filazi (2010). This solution was composed of Acetone-chloroform-n-propanol-impregnation liquid (16 + 20 + 27 + 16).

The impregnation liquid was a pre-prepared solution (0.1 N phthalate, pH 3.75, and glycerin, 19+1).

2.2.2.2 Thin-layer chromatography

The samples that showed a positive result (a clear inhibition zone; 4 pasteurized milk, 12 raw milk) were applied to thin-layer chromatography as described by Kaya and Filazi (2010). In the current study, two TLC plates were used, one for the pasteurized milk sample and the other for the raw milk samples. The TLC plate used for pasteurized milk was divided into 6 equal channels (4 pasteurized milk and 2 standard antibiotics), while the TLC plate used for raw milk samples was divided into 14 equal channels (12 raw milk and 2 standard antibiotics). Ten microliters of the extract were spotted onto a glass capillary tube. The plates were then allowed to dry for 5 minutes at room temperature to prevent sample decomposition. Subsequently, each plate was placed in a TLC tank containing the mobile phase, which had been prepared an hour beforehand to achieve an optimal saturated environment, and the tank was covered with a lid. Once the solution front reached to the line drawn below 1 cm from the up edge, the plates were removed, dried, and then transferred to a freshly tank. Two methods were used to visualize the spots: UV detection box at 254 nm (Skorupa and Gierak, 2011; Hayati and Anggraini, 2023) and evaporated Iodine. To calculate the Retardation factor (Rf), sample spots were marked with a pencil. The Rf value is determined using Equation 2 (Kumar *et al.*, 2013).

$$Rf = \frac{distance\ travelld\ by\ component}{distance\ traveled\ by\ solvent} \quad \text{(Eq. 2)}$$

A matching Rf value between a sample and a standard indicates that two compounds are identical (Fink *et al*.,1963; Cieśla *et al*.,2009)

2.2.2.2 Quality control

The limit of detection (LOD) and the limit of quantification (LOQ) to evaluate the sensitivity of TLC for penicillin G and streptomycin, a serial dilution was prepared. Different concentrations (50, 25, 10, 5, 1, and 0.5 μg/mL) were prepared by dissolving the standard antibiotics in methanol to obtain the desired concentrations. These concentrations were used for minimum detectable concentrations, as mentioned by Kaya and Filazi (2010).

The recovery (%) of an antibiotic with a milk sampling was detected by adding a known concentration of (50, 25, 5 μg/mL) of standard antibiotic to a milk-free antibiotic sample. Followed by extracting the antibiotic from milk as described by Tyczkowska *et al*. (1989), one mL of the milk sample was mixed with 1 mL of protein precipitation solution (acetonitrile-methanol-deionized water at a ratio of 40:20:20, respectively) in a centrifuge tube. After gently shaking the tube, it was centrifuged at 3000 rpm for 10 minutes. Then spiking the samples in the TLC plate. Recovery (%) was calculated using Equation 3.

$$Recovery\ (\%) = \frac{\text{Amount detected after extraction}}{\text{Amount of originality added}} \times 100 \quad \text{(Eq. 3)}$$

The detection of Precision (RSD%) of the TLC method was evaluated by performing replicate analyses (n=4) of spiked milk samples with a known concentration of antibiotic (25, 10, and 5 μg/mL. The RF value was measured for each run. The RSD% was calculated using Equation 4. RSD% values below 5% are considered good precision.

$$RSD\ (\%) = \frac{\text{SD}}{\text{Mean}} \times 100 \quad \text{(Eq. 4)}$$

2.3 Statistical Analysis

SPSS version 20 software for descriptive statistical analysis was used for results analysis. To evaluate the prevalence of antibiotic residues, milk samples were categorized as 'positive' or 'negative' based on the presence of an inhibition zone ≥2 mm. Given the unequal group sizes (n=13 vs. n=32) and the presence of small expected frequencies in some categories, **Fisher's Exact Test** was employed to determine if the proportion of positive antibiotic detections differed significantly between raw and pasteurized milk. This approach provides a robust, non-parametric assessment of the association between milk processing type and the presence of veterinary drug residues. Results were considered statistically significant if $P < 0.05$. Also, the Confidence Interval (CI) at 95% was calculated

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Antibiotic sensitivity test for *B. subtilis*

B. subtilis HS was found to be sensitive to all the antibiotics tested. The results, shown in **Table 1**, demonstrated its susceptibility to kanamycin, neomycin, streptomycin, ampicillin, amoxicillin, penicillin G, ciprofloxacin, chloramphenicol, and tetracycline. Because of this broad sensitivity, *B. subtilis* HS is a strong candidate for use as a test microorganism to detect antibiotic residues in milk.

3.1.2. Detection of antibiotic residue in milk using the Agar well diffusion method

The results of the Agar well diffusion method for detecting antibiotic residues showed that, out of 13 pasteurized samples collected from markets, only 4 (30.76%) gave a positive result (formation of an inhibition zone around the holes). Of 32 raw milk samples, only 12 (37.5%) tested positive for antibiotic residues. Altogether, the milk samples showed a total occurrence of antibiotic residues of 35.5%, as presented in **Table 2.** The positive and negative results for the antibiotic residue in milk are shown in **Figure 1.**

The diameter of the inhibition zone was varied between pasteurized and raw milk **Figure 2.** The raw milk showed a larger zone of inhibition than pasteurized milk. Even within the same group, the inhibition zone was varied**.** However, there was no significant difference between

pasteurized and raw milk ($p<0.05=0.52$). Statistically, the 95% confidence interval for both pasteurized and raw milk samples was 14.75%-22.59%.

The minimum inhibition zone diameter for antibiotic residues in milk was detected using Agar well diffusion method at concentrations of 25, 10, 5, 2, and 1 μg/mL). Results in **Table 3** and **Figure 3** showed that the minimum inhibition zone diameters for Penicillin G and Streptomycin were 10 $\mu l/mL$ and 5 $\mu l/mL$, respectively.

3.1.3. Detection of Penicillin G and Streptomycin antibiotic residue in milk using the TLC method

The milk samples that showed a positive result (inhibition zone) were subjected to a TLC test to identify the antibiotic residue present in the milk. Penicillin G and Streptomycin were used as standard antibiotics. As shown in **Table 4**, the Retardation factors (Rf) were varied between and within the milk samples. For the presence of Penicillin G residue, there were 9 milk samples (56.25%), 1 belonging to pasteurized milk; P11 and 8 belonging to raw cow milk; C3, C8, C12, C17, C18, C22, C26, and C30 showed an identical spot with the standard antibiotic penicillin G at Rf value 0.85. Regarding the presence of Streptomycin residue, 2 samples (12.5%) from raw cow milk (C29 and C34) showed an identical spot with the standard antibiotic streptomycin at an Rf of 0.51. On the other hand, the combination of Penicillin G and Streptomycin antibiotic residues was detected in 2 samples (12.5%) from raw cow milk (C1 and C20), which showed 2 spots identical to the standards. However, 3 samples from pasteurized milk (P3, P6, and P7) showed spots that were not identical to the standard antibiotic, with different Rf values.

The precision test (RSD%) values for Penicillin G and Streptomycin are shown in **Table 5**. Where the RSD% for penicillin G was 1.7% and for streptomycin was 2.3%.

3.1.4. Quality control

The sensitivity of the TLC method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) for Penicillin G and Streptomycin in milk. The LOD values for Penicillin G were found to be 0.5 μg/mL as shown in **Table 6** and **Figure 4**. For Streptomycin, the LOD values were found to be 1 $\mu g/mL$ as shown in **Table 6** and **Figure 5**. The LOQ values for Penicillin G and Streptomycin were 1.5 $\mu g/mL$ and 3 $\mu g/mL$, respectively (**Table 6**).

The recovery % for Penicillin G was 82-87%, while for Streptomycin was 85-89%, which are considered good values, **Table 6.**

3.2. DISCUSSION

Since Alexander Fleming discovered antibiotics, millions of lives have been saved. Despite that, the future of antibiotics used in medicine is more complicated than anyone expected (Hutchings *et al*., 2019). The overuse of antibiotics contributes to the development of antibiotic-resistant strains. This resistance is based on the concept of Darwinian survival of the fittest (Fleming, 2006). The use of antibiotics is not limited to human pathogenic infections; it also includes animal and plant infections (Arsène *et al*., 2022). Reports of antibiotic residues in milk began appearing in the 1960s, but detections significantly rose after 2000. This issue has drawn considerable attention recently, driven by growing concerns over food safety and public health (Sachi *et al*., 2019). The presence of antimicrobial residues in milk and dairy products is considered a serious threat to public health (Ghimpeţeanu *et al*.,2022).
Therefore, simple, and effective screening tests are important for identifying residues of various classes of antimicrobials in food, particularly milk (Islam *et al*., 2020). In the current study, the occurrence of antibiotic residues in raw milk samples collected from the fields using Agar well diffusion method was higher (37.5%) than in pasteurized milk samples collected from markets in Karbala (30.76%). This could be attributed to several reasons; first, farmers do not follow the withdrawal periods set by the Codex Alimentarius Commission after administering medication to their animals, which can result in contamination of their raw milk (Alimentarius, 2010). As is well known, withdrawal periods refer to the time required for an animal to fully metabolize and eliminate a given antibiotic from its tissues, thereby reducing it to a safe and acceptable level for consumption (Virto *et al*., 2022). Each antibiotic has a specific withdrawal time, such as Penicillin 72 hours, Amoxicillin 60 hours (Burmańczuk *et al*., 2017), and a combination of

Streptomycin with Penicillin 72 hours (Karande *et al*., 2021). Accordingly, milk produced during that time must be disposed of. Secondly, the lack of control over raw milk sales, where farmers sell raw milk directly without undergoing strict testing or meeting quality control standards (Zavala and Revoredo-Giha, 2022). Unlikely, the milk intended for pasteurization is usually tested for antibiotic residues at collection centers; milk exceeding safe limits is rejected, ensuring that the raw milk used for pasteurization has very low or undetectable levels of these residues (Rahman *et al*., 2021).

To sum up, the greater detection of antibiotic residues in raw milk compared to pasteurized milk is largely due to residues originating from farm-level veterinary drug use. However, the pasteurization process, along with the quality control measures in place for milk intended for pasteurization, degrades or inactivates some of these residues, resulting in lower levels in the final product. Despite that, the percentage of total occurrence of antibiotic residue in the current study was 35.5%, which was concordant with other studies with a total occurrence ranging from 28-35%, such as Iran 34% (Olatoye *et al*.,2016), Kenya 30.7% (Orwa *et al*., 2017), and Somalia 30% (Mohamed *et al*., 2020). Although many studies have detected antibiotic residues in milk samples, the overall incidence has varied. This variation was low in some regions (Kumarswamy *et al*., 2018) and high in others (Stella *et al*., 2020). This variation could be attributed to many factors, such as withdrawal time of milk, the method used to detect antibiotic residues, and the stability of the antibiotic in milk (Sachi *et al*., 2019). Also, the experimental season was conducted. A study by Moghadam *et al*. (2016) showed that the winter season had a higher incidence of antibiotic residues in milk than the spring season. Likewise, this study was conducted during the winter season when antibiotic residues are typically more common. This can be attributed to colder temperatures and shorter days, which lead animals to be kept indoors more often, increasing the risk of infections and, in turn, prompting the use of an antibiotic for treatment (Alimohammadi *et al*., 2020).

In the current study, the choice of *B. subtilis* to detect penicillin G and streptomycin residues in milk samples is due to its remarkable sensitivity to these antibiotics (Titouche *et al*., 2022). This led to the next part of the work, which was the use of penicillin G and streptomycin as a standard for performing TLC tests on samples that showed a positive result. Table 2 clearly demonstrated that penicillin G had the highest antibiotic residue in milk samples (56.25%), followed by streptomycin (12.5%) and the combination of penicillin G and streptomycin (12.5%). The reasons for the higher occurrence of penicillin G as an antibiotic residue in milk compared to streptomycin are that penicillin G belongs to the beta-lactam antibiotics, known for its wide spectrum of activity against gram-positive pathogens such as streptococcal and staphylococcal infections (Okonko *et al*., 2009). Also, penicillin G has fewer side effects than streptomycin, which can cause an allergic reaction in cattle (Hirvonen *et al*., 1994). Additionally, the low cost, availability, and effectiveness of treatment for infectious diseases lead farmers in remote areas to use penicillin G without veterinary guidance (Layada *et al*., 2016; Batah *et al*., 2025). In general, this study agrees with other studies that reported a high rate of penicillin G in milk samples compared to other antibiotics (Kaya and Filazi, 2010; Malgwi *et al*., 2023).

Numerous analytical techniques exist for detecting antibiotic residues in milk, including microbiological tests, chromatographic methods, immunochemical assays, and receptor- and enzyme-based tests (Kantiani *et al*., 2009; Kaya and Filazi, 2010). Among these, microbiological tests, particularly the agar well diffusion method, are frequently used in the dairy industry to detect antibiotic residues (Titouche *et al*., 2013; Almashhadany, 2021). The combination of thin-layer chromatography (TLC) with microbiological detection methods, such as the agar well diffusion assay, is an effective approach for detecting antibiotic residues in milk. This method is valued for its simplicity, low cost, sensitivity, and specificity in identifying various antibiotics (Kaya and Filazi, 2010; Piech *et al*., 2016).

At this point, robust disease prevention practices should be implemented to minimize the need for antibiotic overuse in livestock. Three main pillars are considered cornerstones for protecting livestock health: smart husbandry, biosecurity, and hygiene (Paramitadevi *et al*., 2023; Jimenez *et al*., 2023). Smart husbandry, using technology to control and optimize all environmental conditions inside livestock houses, is ideal for controlling disease spread. An example of smart husbandry is the use of sensors to adjust ideal temperature, humidity, and ventilation levels (Ongom, 2023), smart

feeding by balancing the quantity of feed that meets the demands of animals (Makkar, 2016), and utilizing sensors to track animal health, behavior, and productivity (Kistanova *et al*.,2024). The biosecurity sector involves restricting and controlling antibiotic prescribing and vaccinating animals (Renault *et al*., 2021). To achieve biosecurity, many practices are used today, including food safety, limiting and managing invasive species, and controlling the risk of pathogen transfer (zoonosis) (Subasinghe *et al*., 2023). Hygiene is the third crucial sector for protecting livestock health. To prevent disease, it is important to maintain a clean-living environment, provide clean water and food, and practice good personal hygiene. Regular cleaning and disinfection of housing, equipment, and vehicles are also very effective in reducing disease risk (Jimenez *et al*., 2023).

To mitigate the harmful effects of antibiotic residue in milk, it's crucial to educate farmers about these risks. This could be accomplished by using an alternative antibiotic, such as a vaccine or phage therapy. Also, the use of prebiotics and probiotics in animal feed, and the use of traditional medicinal herbs to reduce the need for antibiotics in foodstuffs are considered promising steps towards minimizing the use of antibiotics.

4. CONCLUSIONS:

This study was conducted in Karbala province, where two types of milk samples were collected. Raw milk from cows was collected from the fields, and pasteurized milk from different brands was purchased from the markets. This study clearly demonstrated that the antibiotic residues in raw milk was higher than in pasteurized milk. The presence of antibiotics in milk is a serious public health concern. This issue stems from the improper use of veterinary drugs and the failure to monitor the withdrawal period - the time required between giving antibiotics to the animal and collecting its milk. This could lead to long-lasting illnesses, which would cause medical costs to go up and high mortality rates. Consequently, this points to inefficient and failing public health rules and to farmers using antibiotics carelessly, which is dangerous for consumers.

5. DECLARATIONS

5.1. Study Limitations

The main limitations of this study were the unclear history of antibiotic use among farmers in Karbala city and the lack of a clear schedule and protocols for veterinarians. For these reasons, the results provide an overall picture of antibiotic residues in milk rather than explaining them in detail. However, the cows in the city are distributed in small groups (fewer than 10 per farmer), so the sample size needs to be large to capture the city's geographic distribution, which is very expensive.

Although TLC is a highly accessible and economical screening method for antibiotic residues, its detection threshold often exceeds the strict MRLs mandated by Codex. Advanced analytical platforms (HPLC/LC-MS/MS) offer superior sensitivity but are significantly more expensive. In this study, TLC was utilized as a viable alternative for preliminary contamination assessment despite these sensitivity constraints."

5.2. Acknowledgments

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5.3. Funding resources

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5.4. Conflicts of Interest

The authors declare that they have no competing interests.

5.5. Data Availability

Raw data are available upon request from the corresponding author (sind.s@uokerbala.edu.iq).

5.6. Author Contributions

The contribution of all authors to this manuscript is summarized as follows:
Shamel Aldorri, Sind: Conception and design, manuscript writing, performed analysis and final approval. Hanan Abdul Kareem Jawad: Data collection. Abd Oun, Hamid Gehad: Conception and design , final approval , critical review of statistics. Islam Ahmed Abd Alsahib: Manuscript writing. Fatima Ahmed Ghashan: Manuscript writing. Khalid A. Hussein: Critical review of statistics and performed analysis. Al-Mashkoor Huda Mohammed: Final approval.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

No artificial intelligence tools were used for data fabrication, statistical analysis, interpretation of results, or scientific decision-making. Textual polishing and grammatical editing were performed using Grammarly.

5.8. Research Integrity Declaration

The authors certify that this research complies with the standards of research integrity, including no data fabrication, no results falsification, no p-hacking or selective reporting, originality, not previously published, and ethical methods.

5.9. Originality & Plagiarism Statement

This manuscript was screened for plagiarism using Turnitin. The overall similarity index was 13%, which is below the acceptable threshold. The authors declare that this manuscript is original work and has not been previously published or submitted elsewhere for consideration. No unattributed text has been copied from other sources, and all references have been properly cited in accordance with APA guidelines. The authors further confirm that this manuscript is not currently under review by any other journal.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

This study utilized commercially available bovine milk purchased from a local retail outlet. As the research involved only food-grade animal byproducts and did not involve direct intervention, experimentation, or interaction with live animals, formal ethical approval from an Animal Care and Use Committee was not required.

6.1. Ethics Committee Approval

Not applicable.

6.2. Informed Consent

Not applicable.

7. REFERENCES:

1. Al-Mashhadany, D. A., Nahla, A. A., Zaki, A. M., & Mohammad, V. S. (2018). Detection of antibiotic residues among poultry meat in Erbil city and impact of thermal processing on

remnants. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, *3*, 237-247. https://doi.org/10.26479/2018.0401.19

2. Alimentarius, C. (2010). Codex alimentarius commission. *Toxicological evaluation of certain veterinary drug residues in food. Disponível em: http://www. codexalimentarius. net/web/jecfa. jsp. Acesso em*, *28*

3. Alimohammadi, M., Askari, S. G., Azghadi, N. M., Taghavimanesh, V., Mohammadimoghadam, T., Bidkhori, M., & Mohammadi, A. A. (2020). Antibiotic residues in raw and pasteurized milk produced in Northeastern Iran were examined using the four-plate test (FPT) method. *International Journal of Food Properties*, *23*(1), 1248-1255. https://doi.org/10.1080/10942912.2020.1800032

4. Allen, H. K., Levine, U. Y., Looft, T., Bandrick, M., & Casey, T. A. (2013). Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. *Trends in microbiology*, *21*(3), 114-119 https://doi.org/10.1016/j.tim.2012.11.001

5. Almashhadany, D. A. (2021). Screening of antibiotic residues in raw milk of cows and buffalos by diffusion assays. *Italian journal of food safety*, *10*(3), 9034. https://doi.org/10.4081/ijfs.2021.9034

6. Amin, M., Rakhisi, Z., & Ahmady, A. Z. (2015). Isolation and identification of Bacillus species from soil and evaluation of their antibacterial properties. *Avicenna Journal of Clinical Microbiology and Infection*, *2*(1), 23233. https://DOI: 10.17795/ajcmi-23233

7. Arsène, M. M. J., Davares, A. K. L., Viktorovna, P. I., Andreevna, S. L., Sarra, S., Khelifi, I., & Sergueïevna, D. M. (2022). The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. *Veterinary world*, *15*(3), 662. https://doi.org/10.14202/vetworld.2022.662-671

8. Back, S.-J.,Park, S.-J.,Moon, J.-S.,Lee, S.-B.,Jo, S.-J.,Nam, T.-J.,Bai, S. C.,Kong, I.-S.,Lee, K.-J.andLee, B.-J. (2020). The effects of dietary heat-killed probiotics bacteria additives in low-fishmeal feed on growth performance, immune responses, and intestinal morphology in juvenile olive flounder paralichthys olivaceus. *Aquaculture Reports*, *18*, 100415. https://doi.org/10.1016/j.aqrep.2020.100415

9. Batah, R., Belbel, Z., Melouah, K., Labied, I., Benaldjia, L., & Berreghis, A. (2025). Detection of antibiotic residues in cow's milk using microbiological screening test in Souk Ahras, Algeria. *Journal of the Hellenic Veterinary Medical Society*, *76*(1), 8865-8870. DOI: 10.12681/jhvms.38195

10. Berendsen, B., Wegh, R., Reinders, T., Lommen, A., & Stolker, A. (2013). Development and validation of a comprehensive LC-MS/MS method for the determination of 46 antibiotics in meat and meat products. *Food Chemistry*, 137(1-4), 160-170.

11. Burmańczuk, A., Tomasz, G., Gbylik-Sikorska, M., Gajda, A., & Kowalski, C. (2017). Withdrawal of amoxicillin and penicillin G procaine from milk after intramammary administration in dairy cows with mastitis. *Journal of Veterinary Research*, *61*(1), 37. https://doi.org/10.1515/jvetres-2017-0005

12. Cháfer-Pericás, C., Maquieira, Á., & Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *TrAC Trends in Analytical Chemistry*, *29*(9), 1038-1049. https://doi.org/10.1016/j.trac.2010.06.004

13. Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M. and Hanage, W. P. (2015). Antibiotics in agriculture and the risk to human health: how worried should we be? *Evolutionary applications, 8*(3), 240-247. https://doi.org/10.1111/eva.12185

14. Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., & Aminov, R. I. (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of environmental quality*, *38*(3), 1086-1108. https://doi.org/10.2134/jeq2008.0128

15. Cieśla, Ł., Skalicka-Woźniak, K., Hajnos, M., Hawrył, M., & Waksmundzka-Hajnos, M. (2009). Multidimensional TLC procedure for separation of complex natural mixtures spanning a wide polarity range; Application for fingerprint construction and for investigation of systematic relationships

within the Peucedanum genus. *Acta Chromatographica*, *21*(4), 641-657. https://doi.org/10.1556/achrom.21.2009.4.10
16. CLSI. M02-A11: Performance Standards for Antimicrobial Disk Susceptibility Tests, 11th ed.; Clincial and Laboratory Standards Institute: Wayne, PA, USA, 2012; Volume 32, ISBN 1562384856.
17. Conceição, S., Queiroga, M. C., & Laranjo, M. (2023). Antimicrobial resistance in Bacteria from meat and meat products: A one health perspective. *Microorganisms*, *11*(10), 2581. https://doi.org/10.3390/microorganisms11102581
18. El Atabani, A. I., El-Ghareeb, W. R., Elabbasy, M. T., & Ghazaly, E. I. (2014). Oxytetracycline residues in marketed frozen beef livers at Sharkia, Egypt. *Benha Veterinary Medical Journal*, *26*(1), 104-112.
19. Ferone, M., Gowen, A., Fanning, S., & Scannell, A. G. (2020). Microbial detection and identification methods: Bench top assays to omics approaches. Comprehensive Reviews in Food Science and Food Safety, 19(6), 3106-3129. https://doi.org/10.1111/1541-4337.12618
20. Fink, K., Cline, R. E., & Fink, R. M. (1963). Paper chromatography of several classes of compounds: correlated Rf values in a variety of solvent systems. *Analytical Chemistry*, *35*(3), 389-398. https://doi.org/10.1021/ac60196a036
21. Fleming, D. O. (2006). Prudent biosafety practices. *Biological Safety: Principles and Practices*, 361-371.
22. Garkavenko, T. O., Gorbatyuk, O. I., Dybkova, S. M., Kozytska, T. G., Andriiashchuk, V. O., Kukhtyn, M. D., & Horiuk, Y. V. (2021). Screening of Epidemiologically Significant Mechanisms of Antibiotics to β-Lactams in Enterobacteriaceae-Pathogens of Zoonoses. *Journal of Pure & Applied Microbiology*, *15*(3). https://doi.org/10.22207/JPAM.15.3.14
23. Ghimpețeanu, O., Tănase, G., Ștefan, C., Vasile, A., & Cătălina, R. (2022). A review of antibiotic residues in food of animal origin and their health implications. *Journal of Applied Animal Research*, 50(1), 384-394.
24. Gondová, Z., Kožárová, I., Poláková, Z., & Mad'arová, M. (2014). Comparison of four microbiological inhibition tests for the screening of antimicrobial residues in the tissues of food-producing animals. *Italian Journal of Animal Science*, *13*(4), 3521 https://doi.org/10.4081/ijas.2014.3521
25. Hayati, E. K., & Anggraini, M. P. (2023). Chemometric Analysis on Fingerprints of Acalypha Indica L Based on the Different Drying Methods. *Walisongo Journal of Chemistry*, *6*(2), 127-133.
26. Hirvonen, J., Pyörälä, S., Heinäsuo, A., & Jousimies-Somer, H. (1994). Penicillin G and penicillin G-tinidazole treatment of experimentally induced summer mastitis—effect on elimination rates of bacteria and outcome of the disease. *Veterinary microbiology*, *42*(4), 307-315. https://doi.org/10.1016/0378-1135(94)90062-0
27. Hou, J., Wan, W., Mao, D., Wang, C., Mu, Q., Qin, S., & Luo, Y. (2015). Occurrence and distribution of sulfonamides, tetracyclines, quinolones, macrolides, and nitrofurans in livestock manure and amended soils of Northern China. *Environmental Science and Pollution Research*, *22*(6), 4545-4554. https://doi.org/10.1007/s11356-014-3632-y
28. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present, and future. *Current opinion in microbiology*, *51*, 72-80. https://doi.org/10.1016/j.mib.2019.10.008
29. Islam, M. A., Sharma, A., Ahsan, S., Mazumdar, S., Rudra, K.andPhillips, C. J. (2020). Welfare assessment of dairy cows in small farms in Bangladesh. *Animals, 10*(3), 394. https://doi.org/10.3390/ani10030394
30. Jechalke, S., Heuer, H., Siemens, J., Amelung, W., & Smalla, K. (2014). Fate and effects of veterinary antibiotics in soil. *Trends in microbiology*, *22*(9), 536-545.
31. Jimenez, C. E. P., Keestra, S., Tandon, P., Cumming, O., Pickering, A. J., Moodley, A., & Chandler, C. I. (2023). Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: a One Health systematic review. *The Lancet Planetary Health*, *7*(5), e418-e434. https://doi.org/10.1016/S2542-5196(23)00049-9
32. Kantiani, L., Farré, M., & Barcelo, D. (2009). Analytical methodologies for the detection of

β-lactam antibiotics in milk and feed samples. *TrAC Trends in Analytical Chemistry*, *28*(6), 729-744. https://doi.org/10.1016/j.trac.2009.04.005

33. Karande, V. K., Jagtap, R., Kalita, D. J., & Ghumare, B. C. (2021). Determination of streptococcal penicillin residues in cow milk after its intramuscular administration. *J Endocrinol Zool Stud*, *9*(2), 151-156.
34. Kaya, S. E., & Filazi, A. (2010). Determination of antibiotic residues in milk samples. *Kafkas Univ Vet Fak Derg*, *16*(Suppl-A), S31-S35. https://DOI:10.9775/kvfd.2009.1174
35. Kistanova, E., Yotov, S., & Zaimova, D. (2024). Intelligent Animal Husbandry: Present and Future. *Animals*, *14*(11), 1645. https://doi.org/10.3390/ani14111645
36. Kumar, S., Jyotirmayee, K., & Sarangi, M. (2013). Thin-layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. *International Journal of Pharmaceutical Sciences Review and Research*, *18*(1), 126-132.
37. Kumarswamy, N. P., Latha, C., Vrinda, K. M., Sethukekshmi, C., & Mercy, K. A. (2018). Detection of antibiotic residues in raw cow milk in Thrissur, India. *Pharma Innov J*, *7*(8), 452-4.
38. Layada, S., Benouareth, D. E., Coucke, W., & Andjelkovic, M. (2016). Assessment of antibiotic residues in commercial and farm milk collected in the region of Guelma (Algeria). International Journal of Food Contamination, 3(1), 19. https://DOI 10.1186/s40550-016-0042-6
39. Lee, J. B., Chung, H. H., Chung, Y. H., & Lee, K. G. (2007). Development of an analytical protocol for detecting antibiotic residues in various foods. *Food Chemistry*, *105*(4), 1726-1731. https://doi.org/10.1016/j.foodchem.2007.05.030
40. Ma, F., Xu, S., Tang, Z., Li, Z., & Zhang, L. (2021). Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health*, *3*(1), 32-38. https://doi.org/10.1016/j.bsheal.2020.09.004
41. Makkar, H. P. (2016). Smart livestock feeding strategies for harvesting triple gain–the desired outcomes in planet, people and profit dimensions: a developing country perspective. *Animal Production Science*, *56*(3), 519-534. https://doi.org/10.1071/AN15557
42. Malgwi, K. D., Umaru, B., Chabri, S. A., Daniel, N., Sanya, L., Maina, U. A., & Saka, S. (2023). Assessment of Oxytetracycline and Penicillin G Residues Levels in Raw and Fermented Milk in Maiduguri, Northeastern Nigeria. *Saudi J Med Pharm Sci*, *9*(3), 140-149. https://doi: 10.36348/sjmps.2023.v09i03.001
43. Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. *Clinical microbiology reviews*, *24*(4), 718-733. https://doi.org/10.1128/cmr.00002-11
44. Moghadam, M. M., Amiri, M., Riabi, H. R. A., & Riabi, H. R. A. (2016). Evaluation of antibiotic residues in pasteurized and raw milk distributed in the South of Khorasan-e Razavi Province, Iran. *Journal of Clinical and Diagnostic Research: JCDR*, *10*(12), FC31. https://doi.org/10.7860/JCDR/2016/21034.9034
45. Mohamed, M. A., Elmi, A. A. S., Dubad, A. B., Hassan, Y. H. S., Osman, A. M., & Bitrus, A. A. (2020). Antibiotic residue in raw milk collected from dairy farms and markets in Benadir, Somalia. *PAMJ-One Health*, *2*(19).
46. Navrátilová, P., Vorlová, L., Dluhošová, S., Bartáková, K., Hanuš, O., & Samková, E. (2024). Screening Methods for Antimicrobial Residues in the Dairy Chain—The Past and the Present. *Antibiotics*, *13*(11), 1098. https://doi.org/10.3390/antibiotics13111098
47. Norris, J. R., & Wolf, J. (1961). A study of antigens of the aerobic sporeforming bacteria. *Journal of Applied Bacteriology*, *24*(1), 42-56.
48. Okonko, I. O., Fajobi, E. A., Ogunnusi, T. A., Ogunjobi, A. A., & Obiogbolu, C. H. (2009). Antimicrobial chemotherapy and sustainable development: the past, the current trend, and the future.
49. Olatoye, I. O., Daniel, O. F., & Ishola, S. A. (2016). Screening of antibiotics and chemical analysis of penicillin residue in fresh milk and traditional dairy products in Oyo state, Nigeria. *Veterinary world*, *9*(9), 948. https://doi.org/10.14202/vetworld.2016.948-954
50. Ongom, J. (2023). Improving Animal Health and Production through Epidemiological Studies and Technological Integration. *International Journal of Scientific*

and Management Research, *6*(07), 279-293. http://doi.org/10.37502/IJSMR.2023.6718

51. Orwa, J. D., Matofari, J. W., Muliro, P. S., & Lamuka, P. (2017). Assessment of sulphonamides and tetracyclines antibiotic residue contaminants in rural and peri urban dairy value chains in Kenya. *International Journal of Food Contamination*, *4*(1), 5. https://DOI 10.1186/s40550-017-0050-1
52. Paramitadevi, Y. V., Priadi, C. R., Rahmatika, I., Rukmana, A., & Moersidik, S. S. (2023). Integration of water, sanitation, and hygiene program with biosecurity: A one health approach to reduce the prevalence and exposure of antibiotic-resistant bacteria in the livestock community. *Int J One Health*, *9*, 181-193. www.doi.org/10.14202/IJOH.2023.181-193
53. Piech, T., Majer-Dziedzic, B., Kostruba, A., Grzelak, E. M., & Choma, I. M. (2016). Thin-layer chromatography—Direct bioautography as an alternative method for screening of antibiotic residues in milk: A comparative study. *Journal of Liquid Chromatography & Related Technologies*, *39*(5-6), 292-297. https://doi.org/10.1080/10826076.2016.1163468
54. Plumb, D. C. (2018). *Plumb's veterinary drug handbook: Desk*: John Wiley & Sons.
55. Prescott, J. F., & Hardefeldt, L. Y. (2024). Other Beta-lactam Antibiotics: Beta-lactamase Inhibitors, Carbapenems, and Monobactams. *Antimicrobial therapy in veterinary medicine*, 169-186. https://doi.org/10.1002/9781119654629.ch9
56. Qian, X., Gu, J., Sun, W., Wang, X. J., Su, J. Q., & Stedfeld, R. (2018). Diversity, abundance, and persistence of antibiotic resistance genes in various types of animal manure following industrial composting. *Journal of Hazardous materials*, *344*, 716-722. https://doi.org/10.1016/j.jhazmat.2017.11.020
57. Rahman, M. S., Hassan, M. M., & Chowdhury, S. (2021). Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh. *Heliyon*, *7*(8). https://doi.org/10.1016/j.heliyon.2021.e07739
58. Renault, V., Humblet, M. F., & Saegerman, C. (2021). Biosecurity concept: Origins, evolution, and perspectives. *Animals*, *12*(1), 63. https://doi.org/10.3390/ani12010063
59. Sachi, S., Ferdous, J., Sikder, M. H., & Hussani, S. A. K. (2019). Antibiotic residues in milk: Past, present, and future. *Journal of advanced veterinary and animal research*, *6*(3), 315. https://doi.org/10.5455/javar.2019.f350
60. Salim, H. M., Huque, K. S., Kamaruddin, K. M., & Haque Beg, A. (2018). Global restriction of using antibiotic growth promoters and alternative strategies in poultry production. *Science progress*, *101*(1),52-75. https://doi.org/10.3184/003685018X15173975498947
61. Sharma, S., Chauhan, A., Ranjan, A., Srivastav, R., Chauhan, R., Singh, V. N., & Jindal, T. (2025). Antibiotic Resistance Patterns in Cold-Adapted Bacillus subtilis From the Southern Ocean and Comparative Genomic Insights. *Frontiers in Bioscience-Elite*, *17*(2), 38809. http://doi.org/10.31083/FBE38809
62. Skorupa, A., & Gierak, A. (2011). Detection and visualization methods used in thin-layer chromatography. *JPC-Journal of Planar Chromatography-Modern TLC*, *24*(4), 274-280. https://doi.org/10.1556/jpc.24.2011.4.1
63. Stella, O. I. O., Ezenduka, E. V., & Anaelom, N. J. (2020). Screening for tylosin and other antimicrobial residues in fresh and fermented (nono) cow milk in Delta state, South-South, Nigeria. *Veterinary World*, *13*(3), 458. https://doi.org/10.14202/vetworld.2020.458-464
64. Subasinghe, R., Alday-Sanz, V., Bondad-Reantaso, M. G., Jie, H., Shinn, A. P., & Sorgeloos, P. (2023). Biosecurity: Reducing the burden of disease. *Journal of the World Aquaculture Society*, *54*(2), 397-426. https://doi.org/10.1111/jwas.12966
65. Tello, A., Austin, B., & Telfer, T. C. (2012). Selective pressure of antibiotic pollution on bacteria of importance to public health. *Environmental health perspectives*, *120*(8), 1100-1106. https://doi.org/10.1289/ehp.1104650
66. Titouche, Y., Akkou, M., & Houali, K. (2022). Detection of antimicrobial residues in bovine meat marketed at Tizi-Ouzou area (Algeria), by a microbial screening assay. *Journal of the Hellenic Veterinary Medical Society*, *73*(2),

Article 2. https://doi.org/10.12681/jhvms.26396

67. Titouche, Y., Hakem, A., Houali, K., Yabrir, B., Malki, O., Chergui, A., ... & Fiţ, N. I. (2013). Detection of antibiotics residues in raw milk produced in Freha area (Tizi-Ouzou), Algeria.
68. Trufanov, O., Kotyk, A., Trufanova, V., Tereshchenko, O., & Zhukorskiy, O. (2015). Detection of antibiotics, active against Bacillus subtilis, in grain and feed. *Agricultural Science and Practice*, *2*(1), 60-66. https://doi.org/10.15407/agrisp2.01.060
69. Tyczkowska, K., Voyksner, R. D., & Aroson, A. L. (1989). Development of an analytical method for penicilling in bovine milk by liquid chromatography with ultraviolet-visible detection and confirmation by mass spectrometric detection. *Journal of Chromatography B: Biomedical Sciences and Applications*, *490*, 101-113. https://doi.org/10.1016/S0378-4347(00)82765-6
70. Vehapi, M., İnan, B., Kayacan-Cakmakoglu, S., Sagdic, O., & Özçimen, D. (2023). Production of Bacillus subtilis soil isolate as biocontrol agent under bioreactor conditions. *Archives of Microbiology*, *205*(1), 52. https://doi.org/10.1007/s00203-022-03381-z
71. Virto, M., Santamarina-García, G., Amores, G., & Hernández, I. (2022). Antibiotics in dairy production: Where is the problem?. *Dairy*, *3*(3), 541-564. https://doi.org/10.3390/dairy3030039
72. Wang, Y., Lu, R., Wang, W., Wang, Q., Hu, X., & Liu, Q. (2022). A review of detection of antibiotic residues in food by surface-enhanced Raman spectroscopy. *Molecules*, 27(22), 7794. https://doi.org/10.1155/2021/8180154
73. Zavala Nacul, H., & Revoredo-Giha, C. (2022). Food safety and the informal milk supply chain in Kenya. *Agriculture & Food Security*, *11*(1), 8. https://doi.org/10.1186/s40066-021-00349-y
74. Zhang, Y., Lu, J., Yan, Y., Liu, J., & Wang, M. (2021). Antibiotic residues in cattle and sheep meat and human exposure assessment in southern Xinjiang, China. *Food Science & Nutrition*, *9*(11), 6152-6161. https://doi.org/10.1002/fsn3.2568

Table 1. *Antibiotic sensitivity test for B. subtilis HS*

Test group	Disc code	concentration	Inhibition zone (mm)	Zone interpretation
Aminoglycosides				
Kanamycin	K	30 µg	32	S
Neomycin	N	30 µg	26	S
Streptomycin	S	10 µg	24	S
β- lactam				
Ampicillin	AM	10 µg	24	S
Amoxicillin	AmC	30 µg	29	S
Penicillin G	P	10 µg	25	S
Fluroquinolones				
Ciprofloxacin	CIP	5 µg	27	S
Phenicol				
Chloramphenicol	C	30 µg	27	S
Tetracycline				
Tetracycline	Te	30 µg	18	S

Table 2. Confidence Interval (CI 95%) and antibiotic residue ocurance for pasteurized and raw milk samples using the Agar well diffusion method depending on the values of the inhibiotion zone (mm)

Parameters	Pasteurized milk	Inhibition zone (mm) as positive results out of 13	Raw milk	Inhibition zone (mm) as positive results out of 32	Total
	P3	5	C1	14	
	P6	5	C3	10	
	P7	4	C8	16	
	P11	6	C12	24	
			C17	10	
			C18	15	
			C20	13	
			C22	12	
			C26	17	
			C29	13	
			C30	12	
			C34	8	
No. of positive samples		4		12	16
95% CI		3.70%-6.30%		11.05%-16.29%	14.75%-22.59%
Antibiotic residue Occurance		30.7%		37.5%	35.5%

Table 3. *The minimum inhibition zone diameter for antibiotic residues in milk was detected using Agar well diffusion method at concentrations (25, 10, 5, 2 ,1 µg/mL) for Penicillin G and Streptomycin. (+), presence of inhibition zone and (-), no inhibition zone.*

Concentration (µg/mL)	penicillin G	streptomycin
25	+	+
10	+	+
5	-	+
2	-	-
1	-	-

Table 4. *Retardation factor (Rf) of pasteurized milk and raw milk samples compared to standard antibiotics, penicillin G and streptomycin, measured by TLC.*

Milk sample	Rf identical with penicillin G[a]	Rf identical with streptomycin[b]	Not identical
P3	-	-	0.29
P6	-	-	0.71
P7	-	-	0.29
P11	0.85	-	-
C1	0.85	0.51	-
C3	0.85	-	-
C8	0.85	-	-
C12	0.85	-	-
C17	0.85	-	-
C18	0.85	-	-
C20	0.85	0.51	-
C22	0.85	-	-
C26	0.85	-	-
C29	-	0.51	-
C30	0.85	-	-
C34	-	0.51	-

a = Retardation factor of standard penicillin G was 0.85
b = Retardation factor of standard streptomycin was 0.51

Table 5. *The Precision test (RSD%) for (n=4) depending on Retardation Factor (Rf) of spiked milk samples (pasteurized and raw milk) for penicillin G and streptomycin.*

Antibiotic	Mean RF	SD	RSD (%)
Penicillin G	0.85	0.015	1.7%
Streptomycin	0.51	0.012	2.3%

Table 6. *Quality control for the TLC test, including LOD, LOQ, and Recovery (%).*

Antibiotic	LOD	LOQ	Recovery (%)
Penicillin G	0.5 µg/ml	1.5 µg/ml	82-87%
Streptomycin	1 µg/ml	3 µg/ml	85-89%

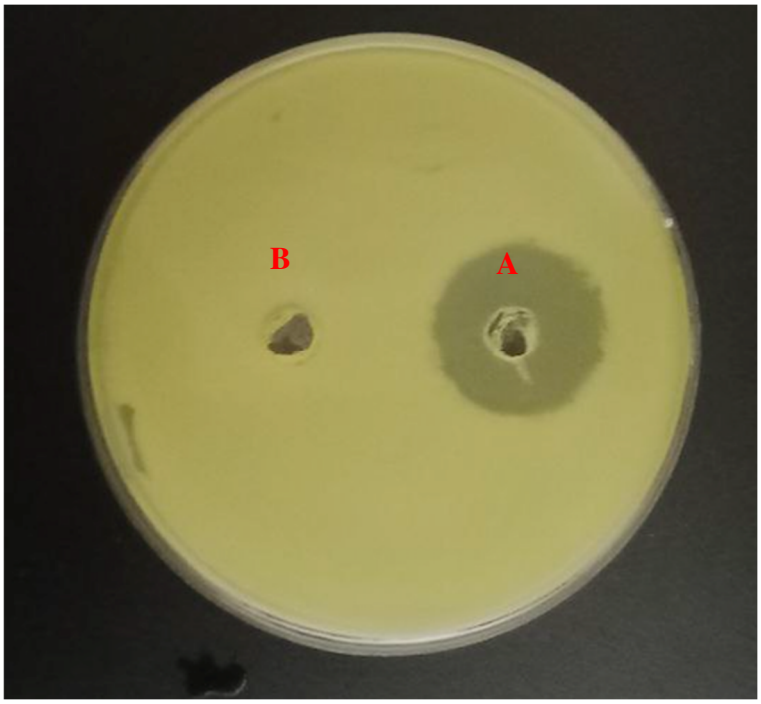

Figure 1. *The inhibition zone (mm) of a milk containing an antibiotic, a positive result (A) and milk without antibiotic, a negative result (B) using the Agar well diffusion method.*

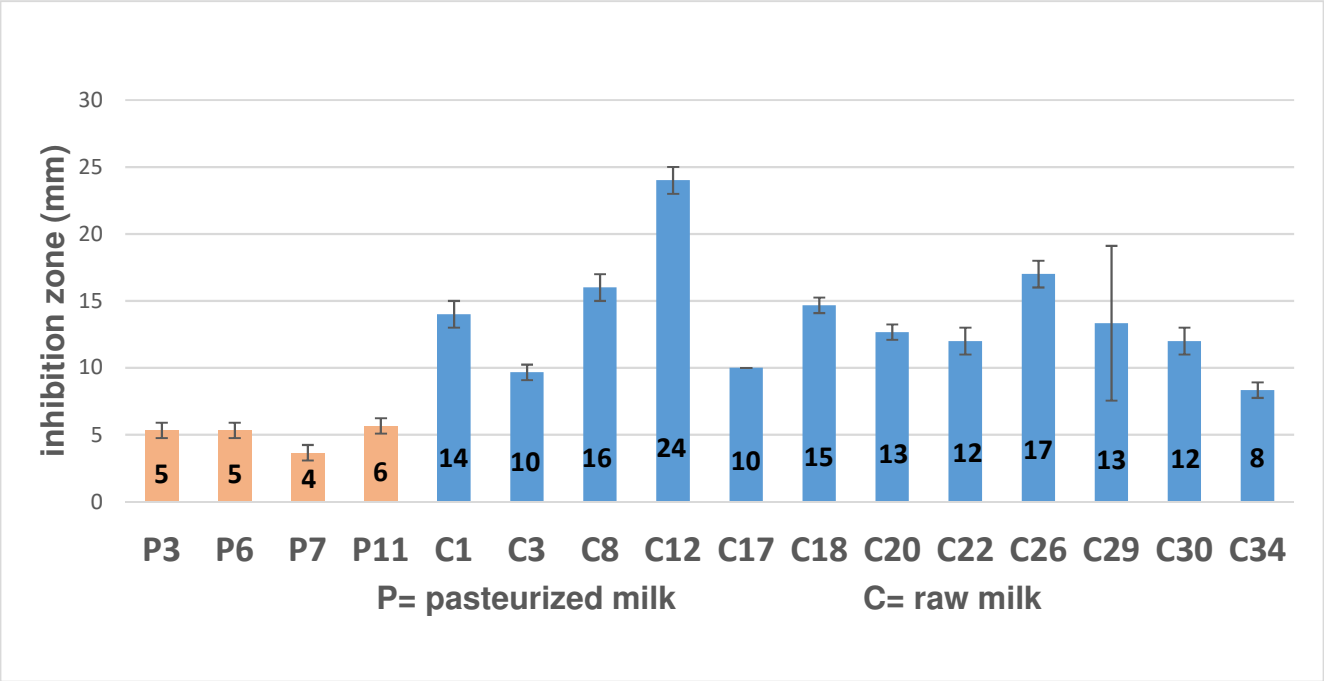

Figure 2. *The dimension of the inhibition zone (mm) of pasteurized milk and raw milk using the Agar well diffusion method. P: Pasteurized milk and C: Cow milk*

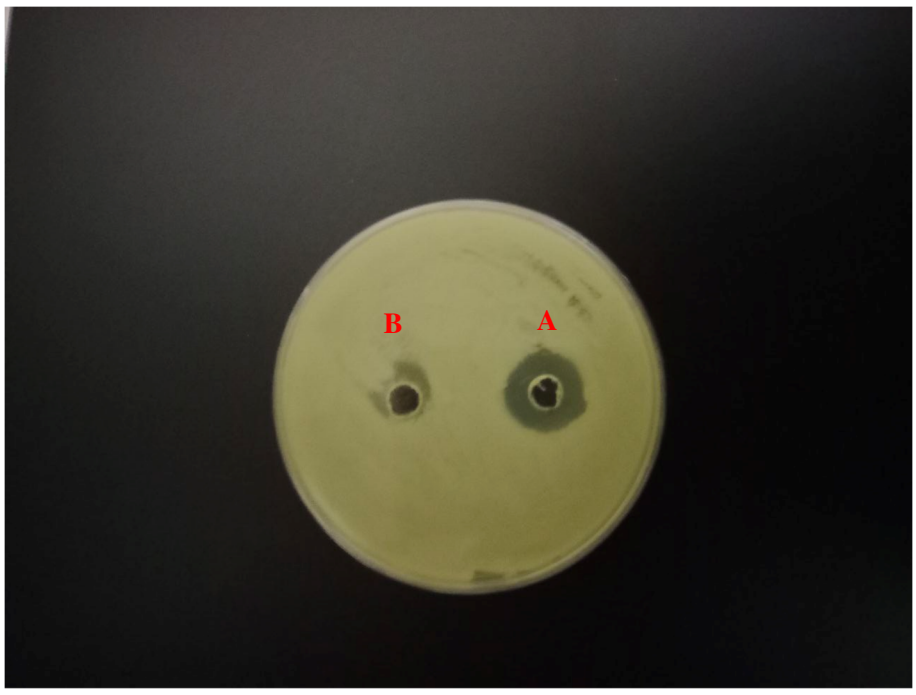

Figure 3. *The minimum inhibition zone diameter for antibiotic residues in milk. (A), penicillin G at concentration 10 µg/mL and streptomycin at 5µg/mL using Agar well diffusion method.*

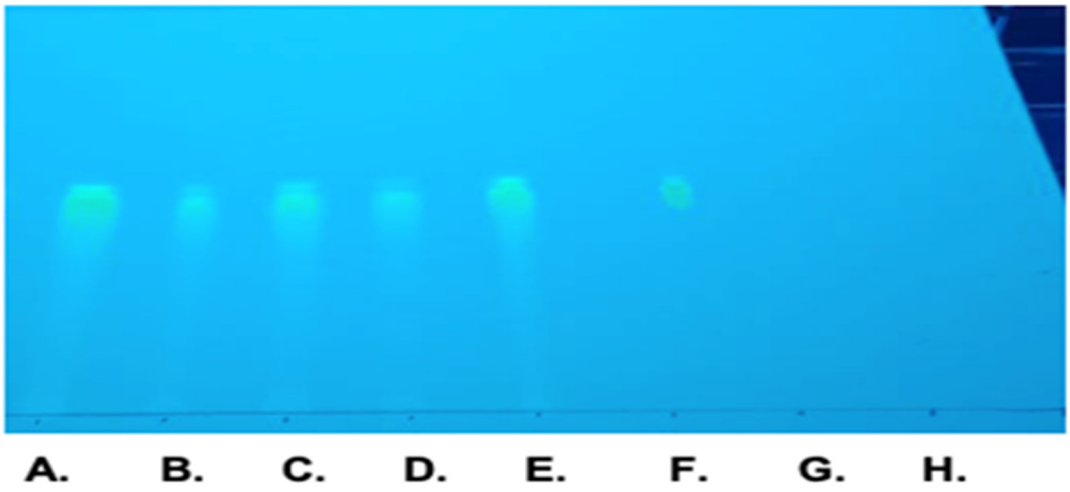

Figure 4. *TLC analysis of penicillin G antibiotic standard at different concentration. Spots corresponding to the following concentration: (A) 100 µg/mL, (B) 50 µg/mL, (C) 25 µg/mL, (D) 10 µg/mL, (E) 5 µg/mL, (F) 1 µg/mL, (G) 0.5 µg/mL and (H) milk-free antibiotic extract used as a negative control.*

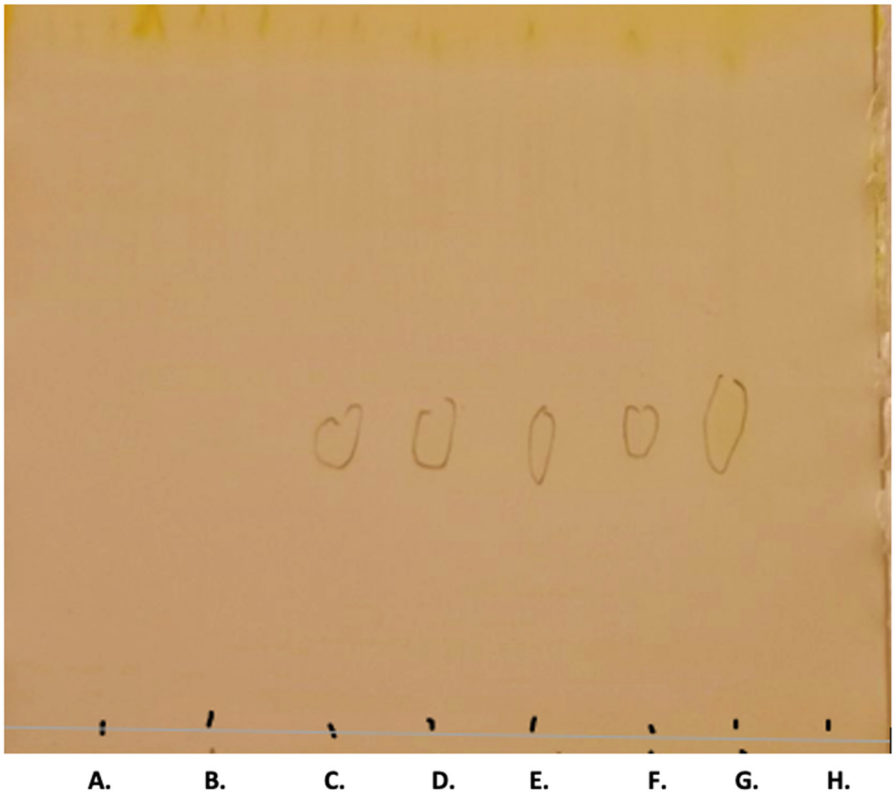

Figure 5. *TLC analysis of streptomycin antibiotic standard at different concentration. Spots corresponding to the following concentration: (A) milk-free antibiotic extract used as a negative control, (B) 100 µg/mL, (C) 50 µg/mL, (D) 25 µg/mL, (E) 10 µg/mL, (F) 5 µg/mL, (G) 1 µg/mL and (H) 0. µg/mL.*

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

ESTRATÉGIA DE CONTROLE PSO-ANN OTIMIZADA PARA MELHORIA DA QUALIDADE DE ENERGIA EM SISTEMAS HÍBRIDOS DE ENERGIA RENOVÁVEL

OPTIMIZED PSO-ANN CONTROL STRATEGY FOR POWER QUALITY ENHANCEMENT IN HYBRID RENEWABLE ENERGY SYSTEMS

استراتيجية تحكم مُحسَّنة باستخدام خوارزمية تحسين سرب الجسيمات والشبكة العصبية الاصطناعية لتعزيز جودة الطاقة في أنظمة الطاقة المتجددة الهجينة

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RESUMO

Introdução: O rápido crescimento no número de fontes de energia renovável (FER) conectadas à rede elétrica tem gerado um problema significativo de qualidade de energia (QE), com instabilidade de tensão no Ponto de Acoplamento Comum (PAC) devido à sua intermitência. Embora diversos controladores tenham sido propostos para tratar essas questões, um controlador DSTATCOM baseado em Rede Neural Artificial (RNA) para a rede híbrida é comumente utilizado. No entanto, uma RNA autônoma tipicamente perde sua capacidade de manter o controle do DSTATCOM devido à convergência prematura resultante do ajuste subótimo dos pesos durante mudanças repentinas nas condições climáticas. **Objetivo**: Desenvolver um método aprimorado para o ajuste dos pesos de uma RNA, a fim de melhorar sua resposta a variações rápidas na geração de energia renovável, garantindo, ao mesmo tempo, que o controlador opere com êxito sob essas condições. Métodos: Este estudo propõe o uso de um algoritmo de Otimização por Enxame de Partículas (PSO) para otimizar os pesos de ajuste de um controlador RNA-DSTATCOM, visando a regulação da tensão do barramento CC de um Sistema Híbrido de Energia composto por Painéis Fotovoltaicos (PV), Turbinas Eólicas (TE) e geração convencional de eletricidade. O controlador proposto foi implementado e avaliado por meio de simulações em MATLAB/Simulink. **Resultados**: Os resultados foram obtidos a partir de simulações comparando o controlador PSO-RNA proposto com controladores PI e RNA autônomos já existentes. O controlador PSO-RNA apresentou desempenho significativamente superior aos outros dois tipos de controladores em termos de estabilidade do sistema e redução de oscilações. A Distorção Harmônica Total (DHT) medida foi de 2,74% para tensão e 3,37% para corrente, atendendo à conformidade com a norma IEEE 519, além de apresentar uma velocidade de resposta notável e superior na restauração da estabilidade do sistema após eventos de perturbação ou falha. **Discussão**: O desempenho aprimorado do controlador PSO-RNA em comparação com outras soluções é atribuído à capacidade do algoritmo PSO de otimizar eficientemente os pesos da RNA, de modo que o controlador PSO-RNA possa ser mais adaptável às condições variáveis resultantes do uso de fontes de energia renovável. Devido às limitações impostas a este estudo pelos cenários de simulação utilizados, trabalhos futuros incluirão validação física e expansão para sistemas híbridos de maior porte. **Conclusões**: O controlador DSTATCOM baseado em PSO-RNA aqui descrito representa um meio eficiente de aprimorar tanto a qualidade de energia quanto a estabilidade dinâmica de sistemas híbridos de energia renovável.

Palavras-chave: *Rede neural artificial; DSTATCOM; Sistemas híbridos de recursos energéticos (SHRE); Otimização por Enxame de Partículas; Controle PI; Qualidade de energia.*

ABSTRACT

Background: The rapid growth in the number of renewable energy sources (RES) connected to the electrical grid has created a significant power quality (PQ) issue with voltage instability at the Point of Common Coupling (PCC) due to their intermittency. Although several controllers have been proposed to address these issues, an artificial neural network (ANN) based on a DSTATCOM controller for the hybrid grid is commonly used. However, a standalone ANN typically loses its ability to maintain DSTATCOM control due to premature

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convergence caused by suboptimal weight tuning during sudden changes in weather conditions. **Aim**: The goal of this research project is to develop an improved method for tuning the weights of an ANN to enhance its response to rapid changes in renewable energy output while ensuring the controller operates successfully under these conditions. **Methods**: This study proposes using a Particle Swarm Optimization (PSO) algorithm to optimize the ANN-DSTATCOM controller's tuning weights to regulate the DC-link voltage of a Hybrid Power System comprising Photovoltaics (PV), Wind Turbines (WT), and conventionally generated electricity. The proposed controller is implemented and evaluated using MATLAB/Simulink simulations. **Results**: Our results were based on simulations comparing the proposed PSO-ANN controller with existing PI and standalone ANN controllers. The PSO-ANN controller performed far better than the other two controller types in terms of system stability and oscillation reduction. Total Harmonic Distortion (THD) is measured at 2.74% for voltage and 3.37% for current, which meets IEEE 519 compliance while providing a record-breaking, superior response speed that restores system stability after disturbance or fault events. **Discussion**: The improved performance of the PSO-ANN controller compared to other solutions is attributed to the PSO algorithm's ability to efficiently optimize ANN weights so that the PSO-ANN controller can be more adaptable to varying conditions resulting from the use of renewable energy sources. Due to the limitations imposed by the simulation scenarios used, future work will include physical validation and expansion to larger hybrid systems. **Conclusions**: The PSO-ANN-based DSTATCOM controller described here represents an efficient means of enhancing both the power quality and the dynamic stability of hybrid renewable energy systems.

Keywords: *Artificial neural network; DSTATCOM; Hybrid energy resource systems (HRES); Particle Swarm Optimization; PI Control, Power Quality;*

الملخص

الخلفية: أدّى النمو السريع في مصادر الطاقة المتجددة المتصلة بشبكة الكهرباء إلى ظهور مشكلة كبيرة في جودة الطاقة، تتمثّل في عدم استقرار الجهد عند نقطة الربط المشتركة نتيجةً للطبيعة المتقطعة لهذه المصادر. على الرغم من اقتراح العديد من وحدات التحكم لمعالجة هذه المشكلة، إلا أنّ الشبكة العصبية الاصطناعية (ANN) القائمة على وحدة تحكم جهاز التعويض المتزامن (DSTATCOM) للشبكة الهجينة تُعدّ الأكثر استخداماً. ومع ذلك, تفقد الشبكة العصبية الاصطناعية المستقلة عادةً قدرتها على الحفاظ على تحكم DSTATCOM بسبب التقارب المبكر الناتج عن الضبط غير الأمثل للأوزان أثناء التغيرات المفاجئة في الأحوال الجوية. **الهدف**: يهدف هذا المشروع البحثي إلى تطوير طريقة محسّنة لضبط أوزان الشبكة العصبية الاصطناعية لتعزيز استجابتها للتغيرات السريعة في إنتاج الطاقة المتجددة، مع ضمان تشغيل وحدة التحكم بنجاح في ظل هذه الظروف. **طرائق العمل**: تقترح هذه الدراسة استخدام خوارزمية تحسين سرب الجسيمات (PSO) لتحسين أوزان ضبط وحدة تحكم القائمة على ANN-DSTATCOM ، وذلك لتحقيق تنظيم جهد وصلة التيار المستمر لنظام طاقة هجين يتكوّن من الخلايا الكهروضوئية وتوربينات الرياح والطاقة الكهربائية المولدة بالطرق التقليدية. وقد تم تطبيق وحدة التحكم المقترحة وتقييمها باستخدام محاكاة MATLAB/Simulink. **النتائج**: استندت النتائج إلى محاكاة قارنت وحدة التحكم PSO-ANN المقترحة مع وحدات التحكم PI ووحدات التحكم ANN القياسية. وأظهرت وحدة التحكم PSO-ANN أداءً أفضل بكثير من النوعين الآخرين من حيث استقرار النظام وتقليل التذبذبات. كما تم قياس التشوه التوافقي الكلي (THD) بنسبة 2.74% للجهد و3.37% للتيار، وهو ما يتوافق مع معيار IEEE 519، مع تحقيق سرعة استجابة قياسية ومتفوقة لاستعادة استقرار النظام بعد الاضطرابات أو الأعطال. **المناقشة**: يُعزى الأداء المُحسّن لوحدة التحكم PSO-ANN مقارنةً بالحلول الأخرى إلى قدرة خوارزمية PSO على تحسين أوزان الشبكة العصبية الاصطناعية بكفاءة، مما يجعل وحدة التحكم PSO-ANN أكثر قابلية للتكيّف مع الظروف المتغيرة الناتجة عن استخدام مصادر الطاقة المتجددة. ونظرًا للقيود التي فرضتها سيناريوهات المحاكاة المستخدمة في هذه الدراسة، ستتضمن الأعمال المستقبلية التحقق العملي والتوسّع ليشمل أنظمة هجينة أكبر. **الاستنتاجات**: تُمثل وحدة التحكم DSTATCOM القائمة على PSO-ANN، كما وُصفت هنا، وسيلةً فعّالةً لتحسين كلٍّ من جودة الطاقة والاستقرار الديناميكي لأنظمة الطاقة المتجددة الهجينة.

الكلمات المفتاحية : الشبكة العصبية الاصطناعية، جهاز التعويض المتزامن، أنظمة مصادر الطاقة الهجينة، خوارزمية تحسين سرب الجسيمات، متحكم PI، جودة الطاقة.

1. INTRODUCTION:

Today, reliance on Renewable Energy Sources (RES) has become vital for supplying power to underserved populations. Solar and wind energy have generally gained more global attention because they are plentiful and sustainable (Das *et al*., 2022). When connecting RES to the utility grid, challenges arise regarding PQ issues such as voltage sags/swells, THD, and changes in power factor (Sahoo *et al*., 2023; Ranjan *et al*., 2024). These problems are caused by the intermittent nature of RES, which can make the grid as a whole unstable (Habib *et al*., 2025). Power compensation devices that help overcome some of these problems include UPQC, SVC, DVR, and DSTATCOM (Jha & Shaik, 2023). However, DSTATCOM is regarded as the most beneficial of these devices due to its cost-effectiveness, compactness, and efficient reactive power support (Etanya *et al*., 2025). The performance of any power compensation device

is influenced by how the function of the compensator is controlled. On the other hand, while traditional controllers are easy to implement, they struggle to maintain stability over long periods due to fluctuations in renewable energy generation (Choudhury & Kumar, 2024). There is a need for advanced intelligent controllers that respond to changes dynamically and provide high reliability.

Many control techniques have been tested recently to improve the performance of DSTATCOM technology in hybrid systems. Historically, Conventional Controllers (Especially the PI Controller) have been the most common controller types used because they are easy to use and implement (Raju *et al*., 2019). However, the main drawback of Conventional Controllers is that they are difficult to tune the proportional and integral gain parameters to maintain stability under highly nonlinear and dynamic load conditions. Hence, researchers have turned to Fuzzy Logic Controllers (FLC) as a better alternative that does not require an exact mathematical model of the system (Rajshekar *et al*., 2025).

The problem with FLC is that its performance depends on the designer's knowledge of how to define the rule base and membership functions, thereby creating a high computational cost for complex systems. Another improvement in this area has been to use Optimization Algorithms to help fine-tune the traditional controllers' parameters. Khadse and Beohar (2024), Alwaeli *et al*. (2025), Srilakshmi *et al*. (2025), and Hammad *et al*. (2023) used algorithms to significantly improve the transient responses of PI Controllers. Even though these methods provided improved performance, no method exists to provide sufficient adaptability to account for the random variation found in renewable energy resources.

As a result, the emphasis has shifted to using AI techniques, especially ANNs, as they are best at learning and adapting to changes in a system (Bousbai, 2026; Hemalatha & Ramasamy, 2020; Sah & Singh, 2023). The latest research indicates an increasing trend toward hybrid intelligent systems to achieve maximum accuracy. By using optimization algorithms with AI models for training and weight optimization, some researchers have achieved improved control performance (Zaro, 2021). Table 1 shows the literature comparison.

While advancements have been made in control strategies for power electronics, several important gaps remain in the literature. Firstly, there is a lack of a systematic methodology for optimizing ANN architectures. Consequently, they are typically deployed without systematic refinements, resulting in limited stability across the entire system. Often, current research either focuses on PI controllers that do not perform well under nonlinear disturbances or completely replaces them with complex AI-type controllers, thereby imposing a significant computational burden and a high implementation cost without differentiating between critical and non-critical control loops. Secondly, the interaction between regulating the DC-link voltage in RES generation, such as a PV system, and operating DSTATCOMs has received very little research attention; therefore, the potential effects this interaction could have on PCC voltage stability during both transient and dynamic events have not been fully explored.

1.1. Aims

To address these challenges, this study contributes to the field by developing a selective control strategy that replaces conventional controllers in the critical DC-link voltage loops of the PV system and the DSTATCOM with an intelligently optimized ANN. The PSO algorithm is employed to develop the optimal ANN structure and weights in a systematic manner, thereby significantly improving the stability and dynamic response of the PCC voltage.

Moreover, these improvements are achieved with minimal additional system complexity by continuing to use traditional controllers in non-critical loops. Thus, the main contribution of the present research is not only to implement an ANN optimization based on PSO but also to introduce a selective control deployment that balances performance improvement and practical implementation in hybrid renewable energy systems. Comparative simulations are performed to evaluate the effectiveness of the proposed control strategy relative to traditional PI and standard ANN controllers under multiple dynamic operating scenarios, including voltage sag and swell disturbances, simultaneous variations in renewable energy generation sources, and single-line-to-ground faults. The results obtained are used to quantitatively demonstrate the impact of the proposed control strategy on PCC voltage stability and power quality. The remainder of this paper is structured as follows: Section 2 details the System Configuration. Section 3 explains the Proposed PSO-ANN Control Strategy. Section 4 presents and analyzes the Results and

Discussion, and Section 5 provides the Conclusion of the study.

2. MATERIALS AND METHODS:

2.1. Materials

The system being researched is a grid-tie hybrid power generation system that combines renewable energy technologies (PV and WT) with a traditional electrical grid supply. A DSTATCOM is used at the point of connection between the grid and the hybrid system to provide power-quality improvements (through reactive power control) and maintain voltage stability as operating conditions vary. This overall system is modeled and simulated using MATLAB/Simulink. To facilitate the development and evaluation of Intelligent Controllers, a dataset consisting of 9000 sample points was created based on analyzing the steady-state response of the hybrid system when operating with a standard analog PI Controller. This data set is then utilized to train and validate the ANN-based Controllers. The technical systems parameters used in the simulations are summarized in Table 2.

2.2. Methods

2.2.1. System Configuration

The architecture of the hybrid system evaluated in this paper is depicted in Fig. 1. The hybrid system comprises two main renewable energy sources, i.e., a solar PV system and a wind energy conversion system. It provides power to the electric utility grid and support load growth, with the intent of supporting the electric utility grid in its ability to accommodate the increase in electric load demand on the utility grid, taking advantage of the facts that solar and wind energy sources are two of the most reliable and sustainable forms of renewable energy sources (Parija *et al.*, 2019). A DSTATCOM is used to provide voltage regulation at the PCC. The primary purpose of the hybrid system is to determine the interactions among the renewable energy sources and the effectiveness of the control strategy in maintaining PCC stability (Taya *et al.*, 2024). Solar energy systems convert sunlight into electrical energy (solar irradiance), and the amount of electricity produced depends on the intensity of sunlight. Moreover, wind energy systems use wind to extract kinetic energy and generate electricity. The output of wind energy systems also varies with wind speed. Both solar and wind have problems due to fluctuations in sunlight and wind. Therefore, they both contribute to continuous variations in active power, leading to issues with voltage stability, reactive power imbalance, and increased THD at the PCC. A DSTATCOM can help resolve these issues through dynamic reactive power compensation and voltage regulation (Prasad *et al.*, 2026). The DSTATCOM's performance will be based on the controller used to regulate the inverter output, which controls both the inverter output voltage magnitude and phase angle to adjust the amount of reactive power injected into or withdrawn from the grid. The amount of reactive power exchanged with the grid can be expressed mathematically as in Equation 1:

$$Q = \frac{V_1(V_1 - V_2 cos\alpha)}{Z} \quad \text{(Eq. 1)}$$

where $V1$ is the grid voltage, $V2$ is the inverter output voltage, Z is the coupling impedance, and α is the phase angle between the voltages. Proper control of $V2$ and α allows effective voltage regulation and improved power quality. To achieve this in the synchronous dq reference frame, the AC terminal voltage regulation is managed by calculating the error according to Equation 2:

$$V_{err}(n) = V_{ref} - V_{actual}(n) \quad \text{(Eq. 2)}$$

This error is processed by an outer PI controller to generate the reference quadrature current $Iqref$ as Equation 3:

$$Iq_{ref}(n) = Iq_{ref}(n-1) + K_p\{V_{err}(n) - V_{err}(n-1)\} + K_i V_{err}(n) \quad \text{(Eq. 3)}$$

where Kp and Ki are the proportional and integral gain constants. The Iq from the abc to dq conversion is done using Park Conversion on all the supply currents. Then, it analogizes Iq and $Iqref$, and uses that to feed an inner Switched PI current controller that generates Vq (Equations 4 and 5).

$$I_{qerr}(n) = I_{qref}(n) - I_q(n) \quad \text{(Eq. 4)}$$

$$V_q(n) = V_q(n-1) + K_p\{I_{qerr}(n) - I_{qerr}(n-1)\} + K_i I_{qerr}(n) \quad \text{(Eq. 5)}$$

Simultaneously, the stabilization of the DC-link voltage Vdc is critical for maintaining the required inverter performance. The DC voltage error is defined in Equation 6:

$$V_{dcerr}(n) = V_{dcref} - V_{dc}(n) \quad \text{(Eq. 6)}$$

This error is processed by an outer PI controller to generate the reference quadrature current $Idref$ as Equation 7:

$$I_{dref}(n) = I_{derf}(n-1) + K_p\{V_{dcerr}(n) - V_{dcerr}(n-1)\} + K_i V_{dcerr}(n) \quad \text{(Eq. 7)}$$

The Id from the abc to dq conversion is done using Park Conversion on all the supply currents. Then, it analogizes Id and $Idref$, and uses that to feed an inner Switched PI current controller that generates Vd. Equations 8 and 9.

$$I_{derr}(n) = I_{dref}(n) - I_d(n) \quad \text{(Eq. 8)}$$

$$V_d(n) = V_d(n-1) + K_p\{I_{derr}(n) - I_{derr}(n-1)\} + K_i I_{derr}(n) \quad \text{(Eq. 9)}$$

Traditionally, a PI controller is used to DC-link voltage regulation for both the PV system and the grid-connected DSTATCOM due to its simplicity. However, its performance is sensitive to parameter tuning and may degrade under rapid fluctuations in renewable generation. ANNs can improve control, but a standard ANN alone cannot solve many dynamic control problems across a wide range of operating conditions. Therefore, this research proposes an ANN optimized using PSO. The PSO-ANN will provide enhanced system response and power quality for any dynamic control application, as discussed in the subsequent sections of the paper.

2.2.2. Proposed PSO-Optimized ANN Control Strategy

To enhance the dynamic performance of the hybrid system, the PSO algorithm is applied with an ANN control strategy proposed to regulate the DC-link voltage of both the PV system and the DSTATCOM. Accurately and quickly regulating DC voltage is very important for the safe operation of the inverter and for providing sufficient reactive power. To achieve this, the dataset used to train the ANN controller was generated from simulation results of the conventional PI controller under different disturbance conditions. A total of approximately 9,000 sample values were collected from both steady-state and transient responses of the DC link voltage. This sample was split into three portions: 70% for training, 15% for validation, and 15% for testing. The ANN inputs are the DC-link voltage error and its derivative, while the output is the control signal applied to the voltage regulation loop. The PSO algorithm was implemented in two phases to optimize both the ANN architecture and its connection weights. The PSO was configured with a swarm size of 30 particles, a maximum of 100 iterations, acceleration coefficients C1 = 1.5 and C2 = 1.5, and an inertia weight of 0.7. The first phase of optimization used PSO to determine an optimal network topology by calculating the number of hidden neurons that yielded the lowest mean squared error (MSE). This optimal structure consisted of two hidden layers: the first with 18 neurons and the second with 28.

After modifying the network topology, the ANN weights were optimized using PSO to improve convergence and dynamic performance (Figure 2). As shown in Table 3, the final reported MSE provides a good representation of the overall optimization process and shows the minimum error achieved by this combined search for both architecture and weights. The PSO-ANN control is subsequently implemented in the system as the replacement for the conventional

PI Controller. Compared to the fixed-parameter PI controller, the proposed PSO-ANN benefits from better adaptability to different solar irradiance and wind speed conditions. Therefore, this results in quicker DC-link voltage stabilization, less overshoot, and overall improvement to the performance of the DSTATCOM to mitigate power quality disturbances. Fig. 3. summarizes the selection of controls in the proposed methodology.

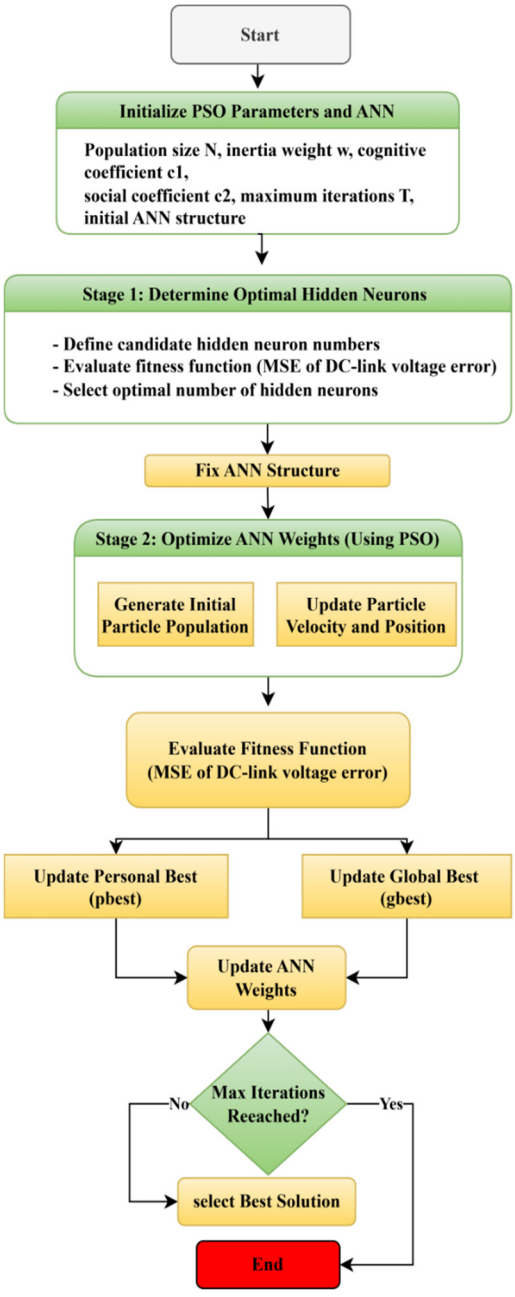

Figure 2. Flowchart of the PSO-ANN algorithm

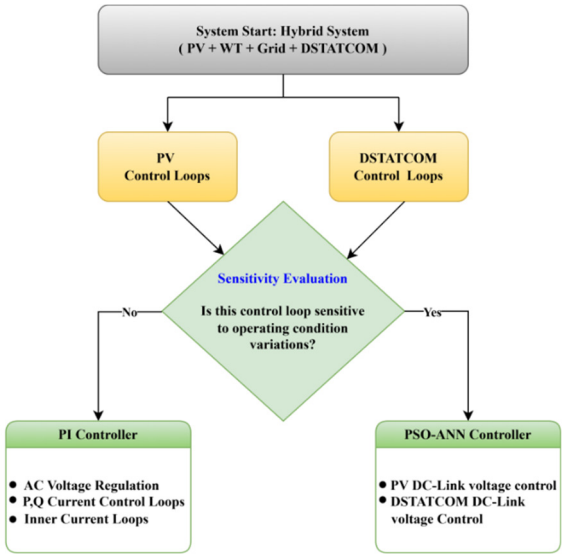

Figure 3 . Proposed hybrid control system.

2.2.3. Controller Tuning and Comparison Setup

To compare the controllers fairly, all controller operations occurred under the same system model, disturbances, and simulation conditions. Conventional PI-controller parameters were tuned using standard methods to achieve stable regulation of the output DC-link voltage under nominal operating conditions, with no variation or noise in the input variables. In contrast, a standard-tuned ANN controller was trained on data generated from the PI-based system's responses, without utilizing PSO optimization techniques. The proposed PSO-ANN controller uses PSO to find an improved set of ANN parameters; therefore, this setup allows for a performance comparison that reflects changes in performance due to differences in the optimization process.

2.2.4. Statistical Analysis

Because the results come from deterministic simulations, no traditional statistical testing will be performed. Assessments will instead be made based on engineering performance metrics. The performance of the controller will be compared with other controllers using standard indices such as THD and the Integral of Time-weighted Absolute Error (ITAE). Both of these indices are commonly used to classify the amount of waveform distortion and the transient response performance of an electrical system.

The ITAE is defined as Equation 10:

$$ITAE = \int_0^T t|e(t)|dt \quad \text{(Eq. 10)}$$

where e(t) is the error between the desired and the actual measured voltage of the DC-link, and T is the total time that the simulation was run. The ITAE penalizes errors that persist for extended periods, which makes it a good indicator of transient response.

The THD is computed as follows:

$$THD = \sqrt{\sum_{n=2}^{\infty} \frac{V_n^2}{V_1}} \times 100\% \quad \text{(Eq. 11)}$$

where V_1 is the fundamental component, and Vn represents the harmonic components of the voltage or current. The THD is used to quantify the degree of harmonic distortion in an electrical system.

All test data were obtained from simulation output files, and none were missing. Lower values of THD and ITAE indicate better power quality and a more rapid dynamic response, respectively.

3. RESULTS AND DISCUSSION:

3.1. Results

The proposed control approach has been demonstrated through simulation results to be capable of addressing power quality issues in grid-interconnected hybrid systems. The proposed controller is well-suited to handle dynamic fluctuations in renewable generation sources and disturbances at the PCC, such as voltage fluctuations and faults, thereby enhancing system stability at the PCC level. Regarding harmonic performance, the proposed controller achieved a THD of 2.74% for voltage and 3.37% for current. Both of these THD values meet the IEEE 519 limits on THD and therefore indicate that the power quality provided to the system during testing met acceptable standards. In addition, the proposed controller provides a much more stable voltage profile and a significantly improved dynamic response than other control methods, as evidenced by reduced oscillations and quicker recovery from disturbances. The core findings from the various testing scenarios are summarized quantitatively in Table 4.

3.2. Discussion

This study assesses both the evaluation of the methodology applied and the effect that the use of this new methodology has on improving power quality and maintaining system stability. The hybrid generation system consists of PV/WT connected to the utility grid, with DSTATCOM connected at the PCC. The simulations are performed in MATLAB/Simulink to evaluate the proposed control strategy against a conventional PI controller and ANN methods. To demonstrate that the proposed control method works across various operating conditions, several scenarios reflecting typical grid-connected power system operation are simulated.

3.2.1. DSTATCOM performance during a voltage swell and sag

Using a programmable AC power source in MATLAB/Simulink, our proposed methodology is tested with voltage sags and swells. A voltage swell occurs from 0.2s to 0.3 s, followed by a voltage sag from 0.3s to 0.4s (as shown in Figure 4(a)), demonstrating how D-STATCOM plays an important role by either absorbing or injecting reactive power into the system to reduce fluctuations caused by these disturbances. When voltage sags occur, the DSTATCOM operates in capacitive mode to inject reactive current into the system and help stabilize the voltage. When voltage swells occur, the DSTATCOM uses inductive mode operation to absorb excess reactive power from the system (acting as an inductor) as shown in Figure 4(b). The effectiveness of reactive power compensation from the DSTATCOM is evidenced by the rapid return to steady-state, with minimal oscillation, of the voltage at the PCC following each disturbance (as shown in Figure 4(c)).

3.2.2. Performance Under Concurrent Dynamic Disturbances

This examination tests the proposed approach's ability to handle multiple simultaneous disturbances and reflects challenging, typical operating environments. The solar radiation decreased rapidly from 1000 W/m² to 800 W/m² at t = 1 s and returned to 1000 W/m² at t = 1.5 s. Parallel to this decrease, wind speed increased instantaneously from 15 m/s to 20 m/s at t = 1.5 s, along with an additional load change at t = 2 s. The voltages at the PCC in Fig. 5 show that the PSO–ANN approach had the least voltage

overshoot and the fastest settling time across all scenarios for voltage control. The PSO-ANN approach also had a greater ability to compensate for reactive power, as illustrated in Fig. 6. In addition to these tests and the voltage sag/swell disturbances, the proposed controller successfully reduced the THD to 3.37% for current and 2.74% for voltage. These values not only exceed the performance of traditional controllers but also comply strictly with the IEEE 519 international standards. Thus, it can be concluded that the proposed system improves power quality and produces a pure sine wave even during many dynamic operational changing conditions.

3.2.3. Performance Under Single-Line to Ground Fault

The testing is designed to confirm the performance of the system and control algorithm in terms of dynamic response to a Single Line-to-Ground (SLG) fault, which commonly occurs. An SLG fault is simulated from t = 0.2 seconds to t = 0.3 seconds to evaluate how quickly the D-STATCOM and controllers can provide reactive power to mitigate the voltage sag caused by the SLG fault and return the system to normal as soon as the fault is cleared. The performance of the system during the pre-fault, fault, and post-fault periods is illustrated in Fig. 7. During the SLG fault, the D-STATCOM effectively alleviates the voltage drop by providing the required reactive power with very good accuracy. The PSO-ANN controller shows a slightly faster recovery from the SLG fault, with a total settling time of 0.01386 seconds after the fault is cleared, while some controllers require approximately 0.014005 seconds to reach steady-state. Although the numerical difference is relatively small, the results consistently indicate that the optimized PSO-ANN controller provides a slightly faster recovery under the studied disturbance conditions.

4. CONCLUSIONS:

In this work, a new integrated DSTATCOM system employing a hybrid PSO-ANN algorithm is proposed to improve power quality in modern power systems. The work included the creation and simulation of a hybrid power system comprising PV, wind, and a conventional source, connected to a utility grid at the PCC. The DSTATCOM is installed as the primary measure for reactive power compensation and voltage stabilization at PCC to help overcome dynamic challenges. The main contribution of this work is the use of a "multiple-selective-level control strategy," in which traditional PI controllers are used for non-critical control loops due to their simple functionality and low processing time. Similarly, the optimized PSO-ANN intelligent controller is positioned to control the most important loops (i.e., the DC Link voltage control that serves both the PV system and the DSTATCOM), critical to ensuring that the entire integrated system operates at an acceptable level of stability. Through comprehensive simulation tests under a range of dynamic conditions, including load changes and faults, the proposed controller demonstrates improved performance compared with conventional PI and standard ANN controllers. The results indicate that a significant reduction in THD to 2.74% for voltage and 3.37% for current is required to be fully compliant with IEEE 519 standards. Future, it shows the ability to quickly restore the stability of the hybrid system after a fault, achieving this within 0.01386 sec, slightly quicker than that of the standard ANN (0.013875 sec) and conventional PI (0.014005 sec) control methods. Thus, the results indicate that the proposed theory provides a computationally efficient means of stabilizing complex hybrid power systems.

5. DECLARATIONS

5.1. Study Limitations

Methodological limitations: This study relies entirely on deterministic simulations performed in MATLAB/Simulink. The system models employ idealized component parameters and do not account for real-world non-idealities such as switching losses, electromagnetic interference, measurement noise, or communication delays between controllers.

Limitations in sample size: The ANN training dataset comprises approximately 9,000 sample points derived exclusively from the steady-state and transient responses of a conventional PI controller. This dataset may not capture the full range of dynamic operating conditions encountered in real hybrid power systems, potentially limiting the generalization capability of the trained network.

Resource or equipment limitations: No Hardware-in-the-Loop (HIL) testing or physical

prototype validation was conducted. All results are based on software simulation, and the actual performance of the PSO-ANN controller in a real-time embedded environment remains unverified.

Generalizability limitations: The proposed controller was evaluated on a single hybrid system configuration (2 MW PV, 2 MW WT, 3 MVAR DSTATCOM, 25 kV grid). Its performance under different system scales, network topologies, higher penetration levels of renewable energy, or alternative renewable sources (e.g., biomass, small hydro) has not been investigated. Additionally, only balanced three-phase load conditions and a limited set of fault types (voltage sag/swell, SLG fault) were considered.

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In accordance with the ethical guidelines of the Periódico Tchê Química, which do not allow donations from authors with manuscripts under evaluation (even when research funds are available), or in cases of authors' financial constraints, publication costs were fully absorbed by the journal under our Platinum Open Access policy, through the support of the Araucária Scientific Association (https://acaria.org/). This policy aims to ensure complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equity in knowledge dissemination.

5.4. Conflicts of Interest

The authors declare no conflict of interest and no competing interests.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author.

5.6. Author Contributions

M.S.A.-O is the main author who conducted the system design and simulations. At the same time, S.D.A.-M. is supervising the research.

5.7. AI and Computational Tools Declaration

The authors declare that they used the generative AI tool Gemini by Google solely to assist with grammar and style improvement and to format figures and tables in this document; any AI-generated outputs were reviewed, verified, and significantly revised by the authors. No AI was used for data generation, statistical analyses, or scientific interpretation of data. The authors retain complete responsibility for the final content of this report and the truthfulness and integrity of this research.

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5.10. Open Access License

6. Ethics Committee Approval

6.1. Ethics Committee Approval

Not applicable (N/A). This research is a simulation-based study using MATLAB/Simu_ link and does not involve any experiments on human participants or animal subjects. Therefore, ethical approval was not required.

6.2. Informed Consent

Not applicable.

7. REFERENCES:

1. Das, S. R., Hota, A. P., Pandey, H. M., & Sahoo, B. M. (2022). Industrial power quality enhancement using fuzzy logic based photovoltaic integrated with three phase shunt hybrid active filter and adaptive controller. *Applied Soft Computing*, *121*, 108762. doi: 10.1016/j.asoc.2022.108762.
2. Sahoo, G. K., Choudhury, S., Rathore, R. S., Bajaj, M., & Dutta, A. K. (2023). Scaled conjugate-artificial neural network-based novel framework for enhancing the power quality of grid-tied microgrid systems. *Alexandria Engineering Journal*, *80*, 520-541. doi: 10.1016/j.aej.2023.08.081.
3. Sarkar, P. R., Minai, A. F., Ahmad, I., Bakhsh, F. I., Khan, A. A., & Pachauri, R. K. (2024). Power quality assessment and enhancement using flc based spv supported cascaded H-bridge multilevel inverter. *e-Prime-Advances in Electrical Engineering, Electronics and Energy*, *7*, 100465. doi: 10.1016/j.prime.2024.100465.
4. Habib, M. A., Arik, M. S. H., & Mostakim, M. A. (2024). AI-Driven Power Quality Analytics and Improvement of Grid Connected Solar Energy Systems. *Journal of Artificial Intelligence General science (JAIGS) ISSN: 3006-4023*, *7*(01), 213-228.
5. Jha, K., & Shaik, A. G. (2023). A comprehensive review of power quality mitigation in the scenario of solar PV integration into utility grid. *e-Prime-Advances in Electrical Engineering, Electronics and Energy*, *3*, 100103. doi: 10.1016/j.prime.2022.100103.
6. Etanya, T. F., Tsafack, P., & Ngwashi, D. K. (2025). Grid-connected distributed renewable energy generation systems: Power quality issues, and mitigation techniques–A review. *Energy Reports*, *13*, 3181-3203. doi: 10.1016/j.egyr.2025.02.050.
7. Choudhury, S., & Sahoo, G. K. (2024). A critical analysis of different power quality improvement techniques in microgrid. *e-Prime-Advances in Electrical Engineering, Electronics and Energy*, *8*, 100520. doi: 10.1016/j.prime.2024.100520.
8. Raju, V. N., & Premalatha, M. (2019). A novel approach for reactive power compensation in hybrid wind-battery system using distribution static compensator. *International Journal of Hydrogen Energy*, *44*(51), 27907-27920. doi: 10.1016/j.ijhydene.2019.08.261.
9. Rajshekar, S., Saikia, L. C., & Nandyala, L. (2025). Optimizing wind energy integration with fuzzy PID DSTACOM and hybrid MPPT for reduced harmonic distortion. *Computers and Electrical Engineering*, *124*, 110391. doi: 10.1016/j.compeleceng.2025.110391.
10. Khadse, D., & Beohar, A. (2024). Enhancement of power quality problems using DSTATCOM: An optimized control approach. *Solar Energy*, *268*, 112260. doi: 10.1016/j.solener.2023.112260.
11. Alwaeli, M. F., Galvani, S., & Talavat, V. (2025). Addressing power quality challenges in hybrid renewable energy systems through STATCOM devices and advanced gray wolf optimization technique. *Results in Engineering*, *25*, 104405. doi: 10.1016/j.rineng.2025.104405.
12. Srilakshmi, K., Gaddameedhi, S., Ramadevi, A., Balachandran, P. K., Reddy, J. G. P., & Vangalapudi, R. (2025). Design and simulation of reduced switch converter based solar PV and energy storage fed shunt active power filter with butterfly optimization. *Results in Control and Optimization*, *19*, 100554. doi: 10.1016/j.rico.2025.100554.
13. Hammad, M. K., & Abdel-naeem, M. A. H. (2023). Power Quality Enhancement of Hybrid PV-wind system using D-STATCOM. *International Journal of Renewable Energy Research (IJRER)*, *13*(1), 504-514. doi: 10.20508/ijrer.v13i1.13662.g8708.
14. Bousbai, K., & Merah, M. (2026). Real-time open-circuit fault diagnosis in wind turbine PWM rectifiers via hybrid THD-enhanced deep learning. *Electric Power Systems Research*, *253*, 112562. doi: 10.1016/j.epsr.2025.112562.
15. Hemalatha, R., & Ramasamy, M. (2020).

Microprocessor and PI controller based three phase CHBMLI based DSTATCOM for THD mitigation using hybrid control techniques. *Microprocessors and Microsystems*, *76*, 103093. doi: 10.1016/j.micpro.2020.103093.
16. Sah, P., & Singh, B. K. (2023). Power quality improvement using distribution static synchronous compensator. *Computers and Electrical Engineering*, *106*, 108599. doi: 10.1016/j.compeleceng.2023.108599.
17. Zaro, F. (2021). Retraction: Voltage Profile Improvement using DSTATCOM Based on Artificial Intelligence Techniques. *Journal of Soft Computing and Artificial Intelligence*, *2*(1), 41-55. https://izlik.org/JA99CF25SE
18. Parija, B., Behera, S., Pattanayak, R., & Behera, S. (2019, March). Power quality improvement in hybrid power system using D-STATCOM. In *2019 3rd International Conference on Computing Methodologies and Communication (ICCMC)* (pp. 564-567). IEEE. doi: 10.1109/ICCMC.2019.8819656.
19. Taya, B. B., Ahammad, A., & Jahin, F. I. (2024). Total harmonic distortion mitigation and voltage control using distribution static synchronous compensator and hybrid active power filter. *International Journal of Advanced Technology and Engineering Exploration*, *11*(114), 624. doi: 10.19101/IJATEE.2023.10102587.
20. Radzi, P. N. L. M., Mekhilef, S., Shah, N. M., Akhter, M. N., Seyedmahmoudian, M., Almalaq, Y., & Wahyudie, A. (2025). Enhancing short-term solar power forecasting using a hybrid deep learning approach and a comparative analysis of machine learning models. *Energy Conversion and Management: X*, 101431. doi: 10.1016/j.ecmx.2025.101431.
21. Jayabarathi, R. (2025). Voltage regulation and stability enhancement in renewable energy micro grids with E-STATCOM under unbalanced loads. *Renewable Energy*, 124716. doi: 10.1016/j.renene.2025.124716.
22. Papana, V. P., Mallala, B., Chinthalacheruvu, V. K. R., & Palle, K. (2025). Evaluating the impact of distribution static compensators on power quality enhancement with finite element interpolated neural networks in modern power systems. *Expert Systems with Applications*, 129214. 10.1016/j.eswa.2025.129214.

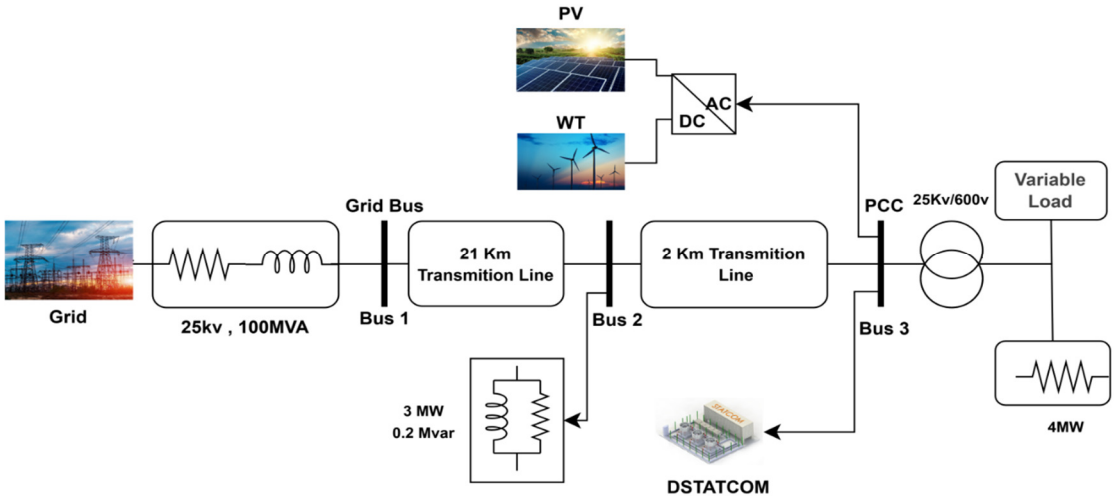

Figure 1. *Proposed hybrid system configuration*

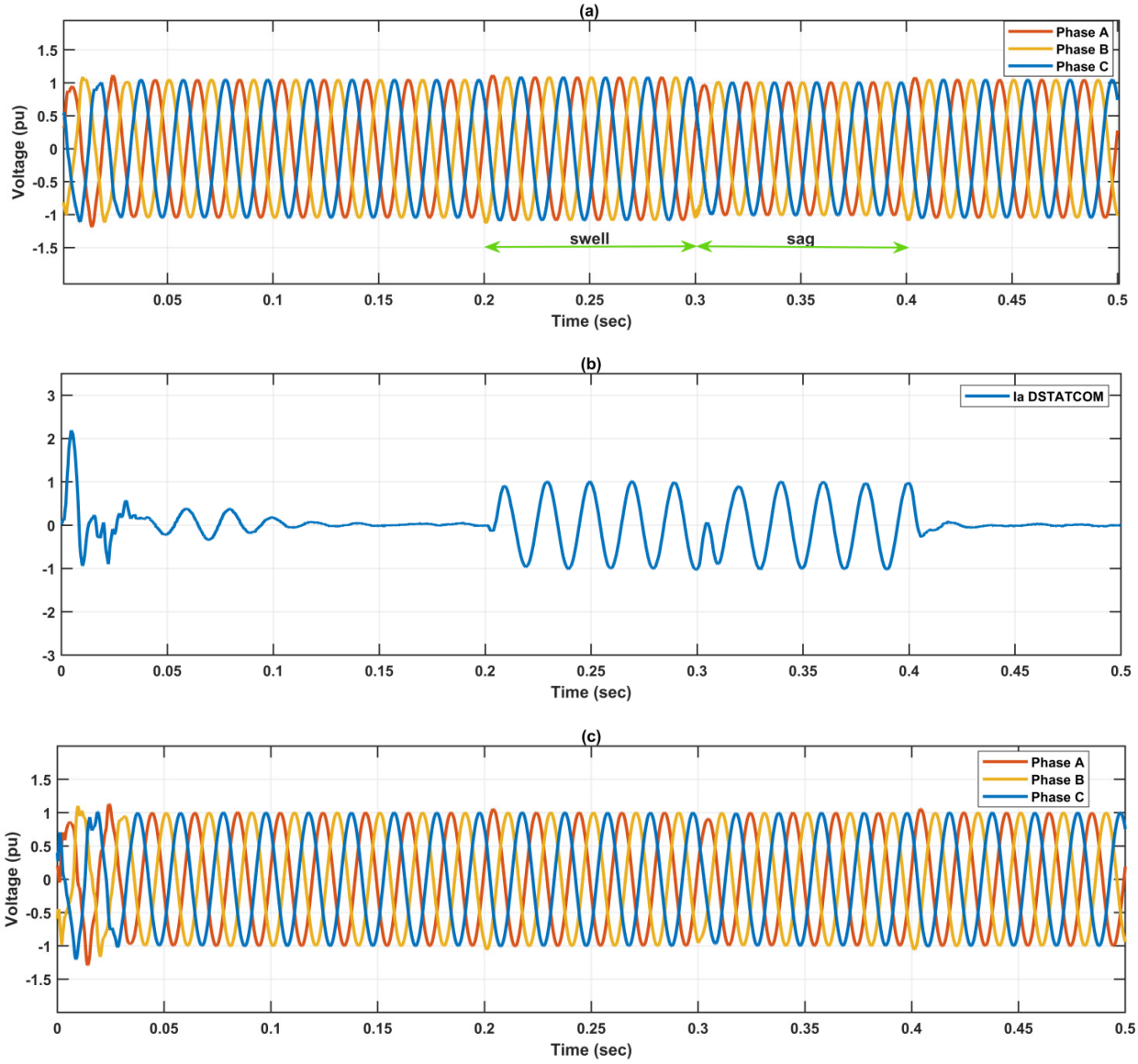

Figure 4. *a) voltage source, (b) injected current, and (c) voltage load*

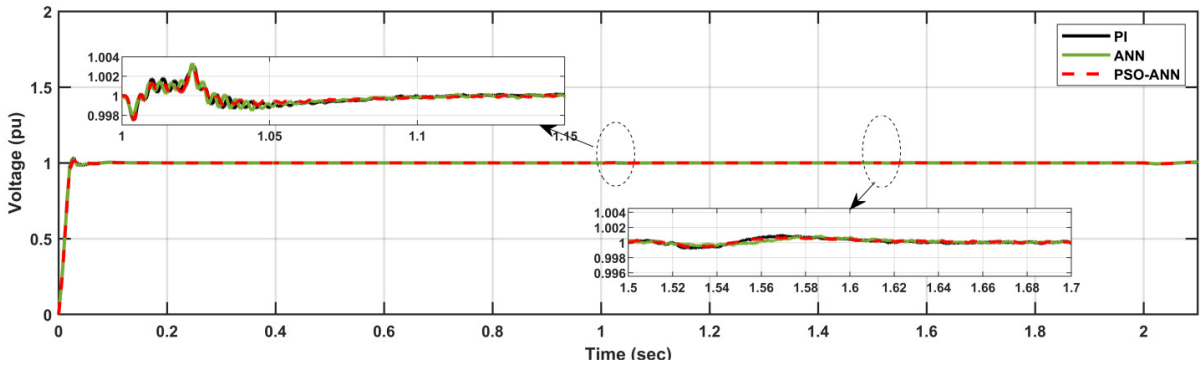

Figure 5. *PCC voltage response comparing three controllers*

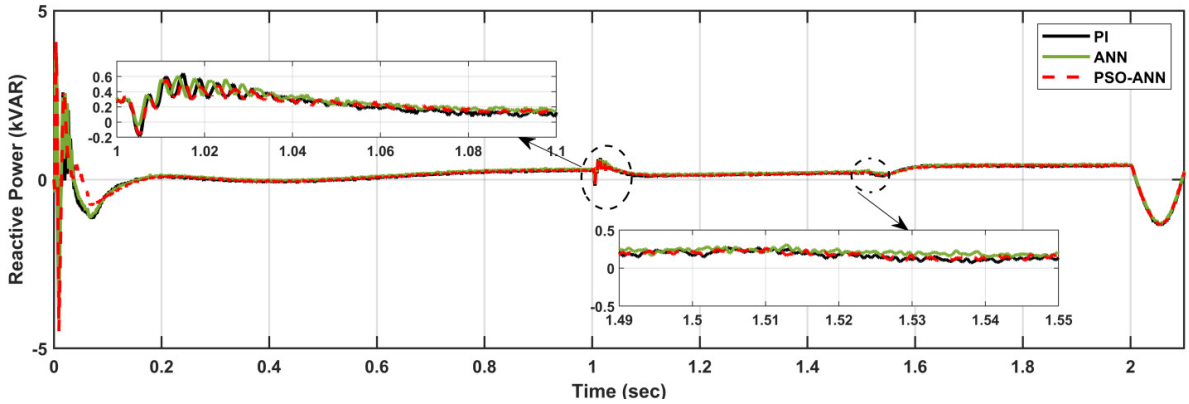

Figure 6. *Reactive Power PCC voltage response comparing three controllers*

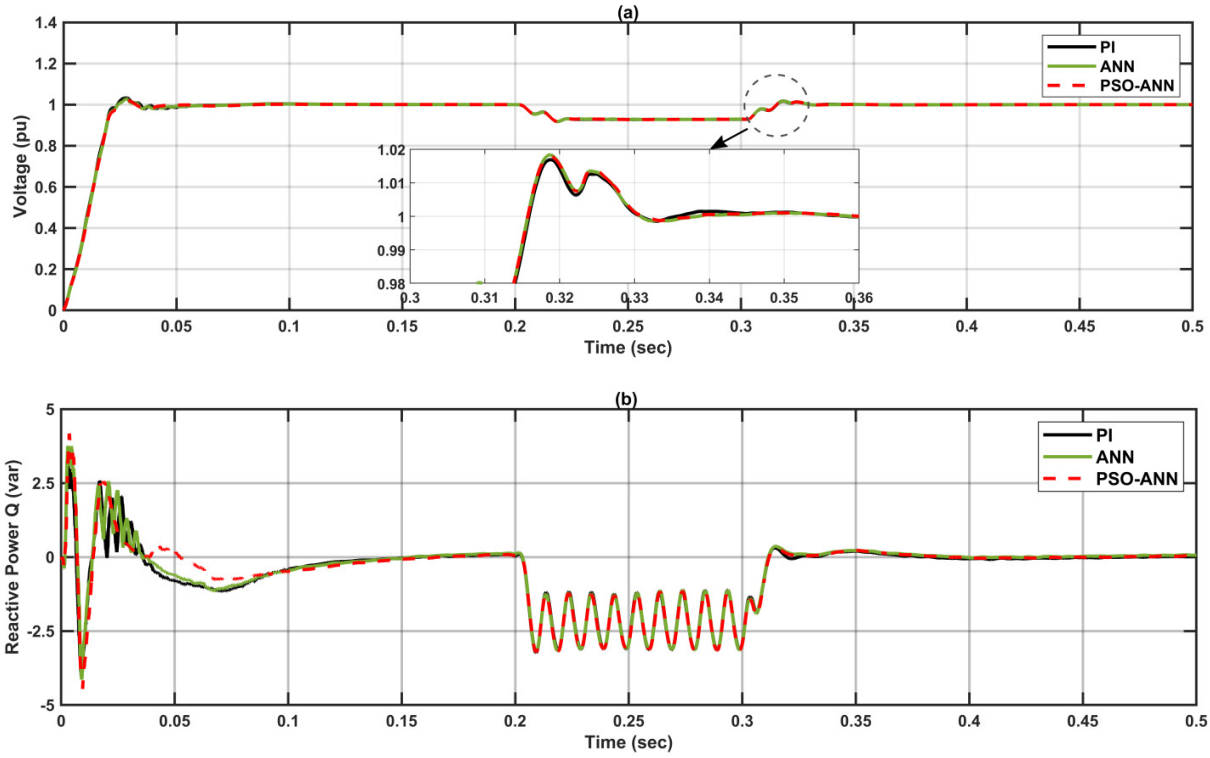

Figure 7. *System performance during SLG fault (a) PCC voltage response in (pu), (b) Reactive power injection for fault recovery*

Table 1. *Literature comparison*

Ref.	Control Method	Key findings	Limitations
(Raju *et al.*, 2019)	PI Controller	Successfully compensated for reactive power, stabilizing the grid through projects completed using PSCAD/EMDTC.	Slow response during disturbances
(Rajshekar *et al.*, 2025)	fuzzy PID-controlled	Reduced grid and load currents' THD to under 0.15% and 0.02%, respectively, while improving MPPT extraction from wind-BESS systems.	Complex rule design
(Alwaeli *et al.*, 2025)	GWO-PI controlled	Maintained PCC within a range of 0.92 and 0.97 pu during three-phase faults, enabling improved dynamic response.	Better than manual PI, but still struggles with nonlinear stochastic solar/wind changes.
(Khadse & Beohar, 2024)	WdCH-PI controlled	Used the Weighted Chimp Optimization method to optimize the fundamental weights of load currents in improving PQ for either balanced or unbalanced loads.	Using traditional distribution networks that have not been validated against the uncertain intermittency of hybrid renewable energy sources (both PV and WT).
(Hemalatha & Ramasamy, 2020)	Standard ANN	Implemented constant DC-link voltage, reduced harm output versus using traditional PI and fuzzy controllers.	Depends on training data quality
(Sah & Singh, 2023)	DBN-CNN	Used Deep Belief Nets and Convolutional Networks to perform advanced levels of harmonic feature extraction for the purpose of reducing harmonic levels.	Cost estimates were based on much larger data sources, and diverse and new scientific methods were used to collect real-time implementation data for this project. A hybrid renewable energy source system has never been utilized within this concept.
Proposed work	PSO-ANN	Successfully balanced ease/low cost of implementation versus accuracy of performance, generating THDv of 2.74% and THDi of 3.37% (per IEEE 519). Achieved high speeds, while minimizing oscillations.	Current validation is restricted to simulation environments. future work requires Hardware-in-the-Loop (HIL) implementation and real-time experimental testing.

Table 2. *Parameters of the System*

	System Parameters	Value
	Main Grid	25 kV,50Hz
PV	Total Capacity	2MW
	DC Voltage set point	1200 V
WT	WT	2MW
	Tr. PV	480 V/ 25 kV
	Tr. WT	575 V/ 25 kV
	Tr. Load	25 kV / 600 V
	Linear load	4 MW, 0.2MVAR; 600 V
DSTATCOM	Source Power	3 MVAR
	DC Voltage set point	2400 V
	Line Resistance	0.12Ω
	Line Inductance	0.0039H
	DC Link Capacitance	10 mF
PI Controller parameters	AC Voltage Regulator	K_P=0.55, K_I=2500
	DC Voltage Regulator	K_P=0.55, K_I=2500
	Current Regulator	K_P=0.8, K_I=200

Table 3. *Training performance comparison between ANN and PSO-ANN*

Algorithm	Hidden (L1)	Hidden (L2)	Total Epochs	Final MSE
ANN	20	-	116	0.0034904
PSO-ANN	18	28	130	0.002443

Table 4. *Comparative System Performance Results*

Performance Parameter	Conventional PI	Standard ANN	Proposed PSO-ANN
THDv	3.15%	3.10%	2.74%
THDi	3.47%	3.46%	3.37%
Settling Time after Fault	0.014005s	0.013875s	0.01386s
ITAE	5.0719e-06	5.0122e-06	4.6594e-06

List of abbreviations

Full From	Abbreviation
Artificial intelligence	AI
Artificial neural network	ANN
Convolutional Neural Network	CNN
Deep Belief Network	DBN
Dynamic Voltage Restorer	DVR
Dynamic Static Compensator	DSTATCOM
Grey Wolf Optimization	GWO
Particle Swarm Optimization	PSO
Photovoltaic	PV
Point of common coupling	PCC
Power quality	PQ
Proportional-Integral	PI
Static VAR Compensator	SVC
Total Harmonic Distortion	THD
Unified Power Quality Conditioner	UPQC
Weighted Chimp Optimization Algorithm	WdCH
Wind Turban	WT

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

RESISTÊNCIA A DROGAS ANTIPROTOZOÁRIAS *EM BLASTOCYSTIS HOMINIS* ENTRE PACIENTES COM TRANSTORNOS MENTAIS E COMPORTAMENTAIS

ANTIPROTOZOAL DRUG RESISTANCE IN *BLASTOCYSTIS HOMINIS* AMONG PATIENTS WITH MENTAL AND BEHAVIORAL DISORDERS

РЕЗИСТЕНТНОСТЬ *BLASTOCYSTIS HOMINIS* К АНТИПРОТОЗОЙНЫМ ПРЕПАРАТАМ У ПАЦИЕНТОВ С ПСИХИЧЕСКИМИ И ПОВЕДЕНЧЕСКИМИ РАССТРОЙСТВАМИ

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RESUMO

Introdução: A resistência antimicrobiana (RAM) é uma ameaça global à saúde pública, com a resistência antiprotozoária recebendo menos atenção, especialmente em populações vulneráveis. Pacientes com transtornos mentais, frequentemente sob terapia psicotrópica de longo prazo, podem apresentar alterações no microbioma intestinal que potencializam infecções parasitárias e resistência a medicamentos. **Objetivo**: Este estudo teve como objetivo investigar a prevalência de parasitoses intestinais e caracterizar a resistência de *Blastocystis hominis* aos antiprotozoários metronidazol e trimetoprima em pacientes com transtornos mentais. **Métodos**: Foi conduzido um estudo retrospectivo com análise de prontuários de 40 pacientes de um centro psiquiátrico. A parasitocenose intestinal foi avaliada por métodos bacterioscópicos e de cultivo em meio de Jones. A sensibilidade de cepas de *Blastocystis hominis* isoladas foi determinada pelo método de disco-difusão e pela concentração inibitória mínima (CIM). **Resultados**: Infecções protozoárias foram detectadas em 27,5% (11/40) dos pacientes, com *Blastocystis hominis* sendo o mais prevalente (53,8% dos casos positivos). Entre as cepas isoladas, 42,9% foram sensíveis ao metronidazol e 57,1% à trimetoprima; as demais foram resistentes. A CIM para metronidazol foi de 10^{-3} mg/mL e para trimetoprima, 10^{-4} mg/mL. **Conclusões**: Foi identificada uma alta taxa de resistência antiprotozoária em *Blastocystis hominis* nesta população vulnerável, possivelmente associada à disbiose intestinal induzida por psicofármacos. Os resultados enfatizam a necessidade de monitoramento parasitológico e teste de sensibilidade antes da terapia empírica nesse grupo.

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Palavras-chave: *Blastocystis hominis; Resistência antiprotozoária; Metronidazol; Trimetoprima; Transtornos mentais; Microbioma intestinal.*

ABSTRACT

Background: Antimicrobial resistance (AMR) is a global public health threat, with antiprotozoal resistance receiving less scrutiny, particularly in vulnerable populations. Patients with mental disorders, often on long-term psychotropic therapy, may have altered gut microbiomes that could predispose them to parasitic infections and drug resistance. **Aim**: This study aimed to investigate the prevalence of intestinal parasitosis and characterize *Blastocystis hominis* resistance to the antiprotozoal drugs metronidazole and trimethoprim in patients with mental disorders. **Methods**: A retrospective study was conducted by analyzing medical records of 40 patients from a psychiatric center. Intestinal parasitocenosis was assessed using bacterioscopic and culture methods on Jones' medium. The susceptibility of isolated *Blastocystis hominis* strains was determined by the standard disk diffusion method and by assessing the minimum inhibitory concentration (MIC). **Results**: Protozoal infections were detected in 27.5% (11/40) of patients, with *Blastocystis hominis* being the most prevalent (53.8% of positive cases). Among the isolated strains, 42.9% were sensitive to metronidazole and 57.1% to trimethoprim; the remaining strains were resistant. The MIC for metronidazole was 10^{-3} mg/mL, and for trimethoprim, 10^{-4} mg/mL. **Conclusions**: A high rate of antiprotozoal drug resistance in *Blastocystis hominis* was identified in this vulnerable population, potentially linked to antipsychotic-induced gut dysbiosis. The findings underscore the need for parasitic monitoring and susceptibility testing prior to empirical therapy in this group.

Keywords: *Blastocystis hominis; Antiprotozoal resistance; Metronidazole; Trimethoprim; Mental disorders; Gut microbiome.*

АННОТАЦИЯ

Введение: Антимикробная резистентность (АМР) представляет собой глобальную угрозу общественному здоровью, причему резистентности простейших уделяется меньше внимания, особенно среди уязвимых групп населения. Пациенты с психическими расстройствами, часто находящиеся на длительной психотропной терапии, могут иметь изменения микробиома кишечника, способствующие паразитарным инфекциям и развитию лекарственной устойчивости. **Цель**: Исследовать распространенность кишечных паразитозов и охарактеризовать резистентность *Blastocystis hominis* к антипротозойным препаратам метронидазолу и триметоприму у пациентов с психическими расстройствами. **Методы**: Проведено ретроспективное исследование на основе анализа медицинских карт 40 пациентов психиатрического центра. Кишечная паразитоценоз оценивалась бактериоскопическими методами и культивированием на среде Джонса. Чувствительность выделенных штаммов *Blastocystis hominis* определялась методом дисковой диффузии и оценкой минимальной подавляющей концентрации (МПК). **Результаты**: Протозойные инфекции выявлены у 27,5% (11/40) пациентов, при этом *Blastocystis hominis* был наиболее распространен (53,8% положительных случаев). Среди выделенных штаммов 42,9% были чувствительны к метронидазолу и 57,1% к триметоприму; остальные штаммы были резистентны. МПК для метронидазола составила 10^{-3} мг/мл, для триметоприма — 10^{-4} мг/мл. **Выводы**: Выявлен высокий уровень резистентности к антипротозойным препаратам у *Blastocystis hominis* в данной уязвимой популяции, что, возможно, связано с индуцированной антипсихотиками дисбиотической средой кишечника. Результаты подчеркивают необходимость паразитологического мониторинга и тестирования чувствительности перед назначением эмпирической терапии в этой группе.

Ключевые слова: *Blastocystis hominis; Антипротозойная резистентность; Метронидазол; Триметоприм; Психические расстройства; Кишечный микробиом*.

1. INTRODUCTION:

The escalating crisis of antimicrobial resistance (AMR) stands as one of the most formidable challenges to global public health in the 21st century, threatening to undermine the foundational pillars of modern medicine. The World Health Organization (WHO) has consistently ranked AMR among the top ten global health threats, warning that without urgent and coordinated action, the world is drifting toward a post-antibiotic era where common infections and minor injuries could once again become fatal (Courvalin, 2016). The ramifications of widespread antimicrobial inefficacy are profound and multifactorial, encompassing increased morbidity

and mortality, prolonged illness duration, higher frequencies of severe complications and bloodstream infections, and staggering economic burdens on healthcare systems and societies worldwide (Mukhina, 2017; Namazova-Baranova & Baranov, 2017). Crucially, the viability of advanced medical interventions—including complex surgeries, organ transplantation, cancer chemotherapy, and management of chronic diseases—is fundamentally contingent upon the ability to prevent and treat infectious complications, a capability critically eroded by the rise of resistant pathogens (Chernenkaya & Godkov, 2015; Kapoor *et al*., 2017).

While the phenomenon of bacterial resistance to antibiotics has justifiably dominated scientific discourse, public health initiatives, and media attention for decades, the parallel and equally critical issue of resistance among eukaryotic pathogens, particularly parasitic protozoa, has received disproportionately less scrutiny. This disparity is concerning given the immense global burden of parasitic diseases, which disproportionately affect populations in regions with limited access to clean water, inadequate sanitation, and fragile healthcare infrastructure (Kappagoda *et al*., 2011). Protozoal infections such as amoebiasis, giardiasis, and blastocystosis contribute significantly to global morbidity, causing diarrheal disease, malnutrition, and growth stunting, particularly in children (Nurtayeva *et al*., 2018). The clinical arsenal of antiprotozoal drugs is inherently limited, and the pipeline for developing novel agents is sluggish and underfunded. Consequently, the preservation of existing therapies through prudent use, robust surveillance, and a deep understanding of resistance mechanisms is not merely important but essential for maintaining global health equity (El-Taweel, 2015).

The human intestinal microbiome, a complex and dynamic ecosystem comprising trillions of bacteria, archaea, viruses, fungi, and eukaryotes, is now recognized as a central orchestrator of host physiology. Its functions extend far beyond digestion to include nutrient metabolism, vitamin synthesis (notably B vitamins and vitamin K), immune system modulation and education, protection against colonization by pathogens (a phenomenon known as colonization resistance), and maintenance of intestinal barrier integrity (Rudzki *et al*., 2021). In recent years, a paradigm-shifting body of evidence has elucidated the existence of a bidirectional communication network between the gut and the brain, termed the gut-brain axis. This axis involves neural, endocrine, immune, and metabolic pathways—including the vagus nerve, the hypothalamic-pituitary-adrenal (HPA) axis, and systemic circulation of microbial metabolites—through which the gut microbiota exerts a significant influence on central nervous system (CNS) function, neurodevelopment, neuroinflammation, and ultimately, behavior (Meng *et al*., 2021; Li *et al*., 2021).

A direct consequence of this understanding is the recognition that a state of imbalance or dysbiosis in the gut microbial community is implicated in the pathogenesis of a diverse array of disorders. These range from gastrointestinal conditions like inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) to systemic metabolic disorders such as obesity and type 2 diabetes, autoimmune diseases, and, most pertinently for this research, neuropsychiatric conditions including major depressive disorder, anxiety disorders, schizophrenia, bipolar disorder, and autism spectrum disorders (Torshin *et al*., 2022; Wang *et al*., 2021). The mechanisms linking dysbiosis to mental illness are multifaceted and interconnected. Firstly, a healthy microbiota is essential for the synthesis and metabolism of key micronutrients, particularly B vitamins (B2, B6, B9, B12), which serve as critical cofactors in the synthesis of neurotransmitters such as serotonin, dopamine, noradrenaline, and gamma-aminobutyric acid (GABA). Deficiencies in these vitamins have been clinically and experimentally linked to the onset and progression of various psychiatric conditions (Rudzki *et al*., 2021). Secondly, dysbiosis can compromise intestinal epithelial barrier function, leading to increased intestinal permeability ("leaky gut") and subsequent translocation of bacterial lipopolysaccharides (LPS) and other pro-inflammatory microbial products into systemic circulation. This triggers a state of chronic, low-grade systemic inflammation and can activate neuroinflammatory pathways in the brain, which is increasingly recognized as a core component of the pathophysiology of several mental disorders (Meng *et al*., 2021). Thirdly, gut microbes themselves produce and modulate a vast array of neuroactive compounds, including short-chain fatty acids (SCFAs), neurotransmitters, and tryptophan metabolites, which can directly or indirectly influence brain function.

A critical and often overlooked factor in this equation is the pharmacological agent itself. It is well-established that broad-spectrum antibiotics cause profound and sometimes long-lasting disruptions to gut microbial diversity and

composition. However, a growing and compelling line of evidence indicates that many psychotropic medications—including antipsychotics, mood stabilizers, and antidepressants—also possess intrinsic antimicrobial properties and can significantly alter the gut ecosystem (Maier et al., 2018; Bugero, 2004). For instance, an influential experimental study demonstrated that administration of lithium, valproate, fluoxetine, or escitalopram to rats over four weeks induced selective and significant shifts in their gut microbiota, notably increasing the relative abundance of bacteria from the Clostridium genus, which includes numerous opportunistic pathogens (Maier et al., 2018). This drug-induced dysbiosis is not a passive side effect but may create a self-perpetuating cycle: psychotropic drugs alter the microbiome, and the altered microbiome, in turn, can affect the drug's pharmacokinetics (metabolism, bioavailability), pharmacodynamics, and even its clinical efficacy and side-effect profile (Li *et al*., 2021). This reciprocal relationship suggests that the gut environment in patients undergoing long-term psychotropic therapy is fundamentally distinct, potentially creating an ecological niche that favors the proliferation of specific microorganisms, including opportunistic parasites.

Among intestinal protozoa, *Blastocystis hominis* occupies a unique position. It is one of the most common eukaryotic organisms found in the human gastrointestinal tract worldwide, with prevalence rates varying dramatically across populations. Blastocystis exhibits significant genetic diversity, with numerous subtypes (STs) identified, of which ST1, ST2, ST3, and ST4 are most frequently reported in humans (Stensvold et al., 2010; Ilyina & Kasatkina, 2010). Its clinical significance remains contentious, ranging from being considered a commensal to a true pathogen associated with irritable bowel syndrome-like symptoms, urticaria, and chronic fatigue (Abedi et al., 2022). The pathogen is transmitted via the fecal-oral route, often through contaminated water or food. Diagnosis traditionally relies on microscopic examination of stained stool smears, though this method lacks sensitivity and specificity compared to modern molecular techniques like polymerase chain reaction (PCR) (Gureser et al., 2023; Rudzińska & Sikorska, 2023).

The therapeutic arsenal for blastocystosis and other protozoal infections is limited. First-line drugs include 5-nitroimidazoles like metronidazole and tinidazole, nitazoxanide, and iodoquinol (Kappagoda *et al*., 2011). However, the efficacy of these drugs, particularly metronidazole, is increasingly compromised by the emergence of resistance. The molecular mechanisms of resistance in protozoa are complex. In related anaerobic protozoa like Giardia lamblia and Entamoeba histolytica, resistance to metronidazole is frequently associated with reduced expression or activity of pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin, enzymes critical for the intracellular reductive activation of the nitro-group of the prodrug into its cytotoxic nitroradical anion (Kapoor *et al*., 2017; Lerminiaux & Cameron, 2019). Data on *Blastocystis hominis* susceptibility are more variable, but studies consistently report that not all clinical isolates are uniformly sensitive, indicating a natural variation and potential for selected resistance (Ilyina & Kasatkina, 2010). Furthermore, the persistence and potential pathogenicity of Blastocystis may be enhanced in a dysbiotic gut environment, as suggested by correlations between its antilysozyme/an_ tilactoferrin activity and the degree of intestinal microbiota disruption (Bugero *et al*., 2020). Recent evidence specifically links *Blastocystis* carriage and metronidazole-resistant forms to patients with schizophrenia, further supporting the relevance of this investigation (Franklin et al., 2022).

Despite the clear intersections between psychotropic drug use, gut dysbiosis, and vulnerability to opportunistic infections, there remains a profound and significant gap in the scientific literature regarding the state of intestinal parasitocenosis—and specifically antiprotozoal drug resistance—in individuals with mental and behavioral disorders. This patient population represents a distinct high-risk cohort: subjected to long-term pharmacological regimens that alter gut ecology, potentially immunocompromised, and often residing in congregate settings like psychiatric hospitals. These factors may collectively foster an environment conducive to the colonization, persistence, and selection of drug-resistant parasitic strains. The scarcity of research addressing this specific nexus constitutes a critical oversight in both psychiatric and parasitological clinical practice. Moreover, regional epidemiological data on intestinal parasites, including resistance patterns, are notably lacking for the Pskov Region of Russia, rendering any localized investigation of substantial public health importance (Nurtayeva *et al*., 2018).

Therefore, this study seeks to bridge this knowledge gap by investigating antiprotozoal drug resistance using a novel and clinically relevant model: the intestinal parasitocenosis of patients undergoing treatment at a specialized psychiatric

institution. By focusing on this pharmacologically manipulated and vulnerable population, the research aims to provide new insights into the ecology of drug resistance beyond the traditional bounds of bacterial pathogens.

1.1. Aims

The primary aim of this study was to identify and characterize resistance to first-line antiprotozoal drugs (metronidazole and trimethoprim) in *Blastocystis hominis* strains isolated from a high-risk cohort—patients with diagnosed mental and behavioral disorders receiving antipsychotic therapy.

The specific objectives derived from this aim were:

1. To determine the prevalence and species composition of intestinal parasites in the studied patient cohort from a psychiatric hospital.

2. To isolate and identify *Blastocystis hominis* from positive samples as the target protozoan for resistance analysis.

3. To evaluate the in vitro susceptibility of the isolated *Blastocystis hominis* strains to metronidazole and trimethoprim using the disk diffusion method.

4. To determine the minimum inhibitory concentration (MIC) of metronidazole and trimethoprim against the isolated *Blastocystis hominis* strains.

2. MATERIALS AND METHODS:

2.1. Materials

The study utilized the following materials, reagents, and equipment to ensure methodological rigor and reproducibility:

Study Population and Data Source: Retrospective data and results were extracted from the anonymized medical records of 40 patients hospitalized at the Pskov Regional Clinical Center for Psychiatry and Narcology between May 2022 and February 2023.

Microbiological Culture Media: Jones' medium, modified, prepared in-house for the isolation and cultivation of Blastocystis hominis. The medium was supplemented with 10% (v/v) inactivated horse serum (Biolot, Russia) to promote protozoal growth.

Staining Reagents: Lugol's iodine solution (Microgen, Russia) for staining stool smears during parasitological examination.

Antibiotic Disks for Susceptibility Testing: Commercially available, standardized antibiotic susceptibility disks (AB Biodisk, Sweden) were used for the disk diffusion assay. The disks contained the following agents and concentrations: Metronidazole (5 µg/disk) and Trimethoprim (5 µg/disk). The potency of each disk lot was verified prior to use.

Antibiotics for MIC Determination: Analytical standard grade Metronidazole (Sigma-Aldrich, USA) and Trimethoprim (Sigma-Aldrich, USA) were used to prepare stock solutions for the broth microdilution tests.

Equipment: Biological safety cabinet class II (Biosan, Latvia); anaerobic workstation with gas mixture (85% N_2, 10% H_2, 5% CO_2) for incubation of Blastocystis cultures (Don Whitley Scientific, UK); binocular light microscope (Olympus CX23, Japan) for microscopy; automatic micropipettes (Eppendorf, Germany); 96-well flat-bottom cell culture plates (Corning, USA) for MIC testing; incubator maintained at 37°C (Binder, Germany).

2.2. Methods

2.2.1 Study Design and Population

A retrospective cohort analysis was conducted. The initial screening identified patients who had undergone mandatory parasitological and bacteriological examination upon admission. The final sample comprised 40 consecutive medical records with complete datasets. Inclusion criteria were: (1) age ≥ 18 years; (2) a primary diagnosis of a mental or behavioral disorder (F00-F99 according to ICD-10); (3) availability of complete results from stool parasitological analysis. All included patients were receiving standard antipsychotic therapy (e.g., haloperidol, chlorpromazine, levomepromazine). Exclusion criteria were not applied based on gender, specific psychiatric diagnosis, or somatic comorbidities to reflect the real-world clinical population of the center

2.2.2 Parasitological Examination and Isolation of Blastocystis hominis

Stool samples were processed according to the methodological guidelines "MUK 4.2.735-99: Parasitological methods for laboratory diagnosis of helminthiases and protozooses." Direct microscopic examination of both native and Lugol's iodine-stained smears was performed to detect cysts and trophozoites of intestinal protozoa. For the isolation and cultivation of Blastocystis hominis, approximately 1 g of stool sample was inoculated into 5 mL of Jones' medium supplemented with 10% inactivated horse serum. Cultures were incubated anaerobically at 37°C for 7–10 days. Culture purity and parasite density were monitored daily by light microscopy. Periods of maximum growth (typically days 3 and 7 post-inoculation), with protozoan concentrations reaching 1–2 × 10^8 cells/mL, were selected for subsequent resistance testing.

2.2.3 Antibiotic Susceptibility Testing

Disk Diffusion Method. The susceptibility of *Blastocystis hominis* isolates to metronidazole and trimethoprim was assessed using an adapted disk diffusion protocol. A standardized inoculum was prepared from a 3-day or 7-day culture, adjusted to a density of 1–2 × 10^8 cells/mL in sterile saline. This suspension was uniformly spread onto the surface of solid agar plates (Jones' medium with 1.5% agar). Antibiotic disks were aseptically placed on the inoculated surface. Plates were incubated anaerobically at 37°C for 48 hours. The diameter of the growth inhibition zone around each disk was measured in millimeters using a calibrated caliper. All tests were performed in duplicate, and the mean diameter was calculated. Laboratory personnel were blinded to any patient identifiers during the reading of inhibition zones.

To ensure methodological validity, quality control was performed using a reference strain of Giardia lamblia (ATCC 30888) with known sensitivity to metronidazole. The inhibition zones for the control strain fell within the expected range for the specific disk lot used.

Interpretation of results for *Blastocystis hominis* was based on criteria adapted from established parasitological research (Ilyina & Kasatkina, 2010; Mirza et al., 2011), as internationally standardized clinical breakpoints (e.g., CLSI, EUCAST) are not currently defined for this organism. Strains were classified as: Highly Sensitive (inhibition zone > 25 mm), Sensitive (15–25 mm), Low Sensitive (10–15 mm), and Resistant (< 10 mm).

Determination of Minimum Inhibitory Concentration (MIC). A broth microdilution method was employed to determine the MIC. Two-fold serial dilutions of metronidazole and trimethoprim were prepared in Jones' liquid medium in a 96-well plate, resulting in a final concentration range of 512 µg/mL to 0.5 µg/mL for both drugs. Each well was inoculated with a standardized culture of *Blastocystis hominis* (final concentration ~1 × 10^5 cells/mL). Positive control wells contained the culture without antibiotics, and negative control wells contained sterile medium only. The plate was incubated anaerobically at 37°C for 72 hours. The MIC was defined as the lowest concentration of the antibiotic that completely inhibited visible growth (no turbidity) compared to the positive control, as assessed by visual inspection under a microscope. Each assay was performed in triplicate.

2.2.4 Statistical Analysis

Data analysis was performed using Microsoft Excel 2019 and R Statistical Software (v4.3.1). Descriptive statistics were applied. Categorical variables (e.g., prevalence of infection, sensitivity/resistance) are presented as absolute counts (n) and percentages (%). Given the sample size, 95% confidence intervals (CIs) for proportions were calculated using the Wilson score interval method to accurately represent the precision of the estimates. Continuous variables (e.g., inhibition zone diameters) are presented as mean ± standard deviation (SD). For comparison of sensitivity rates between the two antibiotics, McNemar's test was applied due to the paired nature of the data (each strain tested against both drugs). A p-value of < 0.05 was considered statistically significant. The reporting of comorbidity prevalence was corrected from mean ± SD to simple percentages with counts (n/N) to avoid statistical error propagation.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Demographic and Clinical Characteristics of the Cohort

The study analyzed data from 40 patients (27 males, 13 females) aged 18 to 65 years (mean age 33 ± 10.5 years). Analysis of medical records revealed the spectrum of comorbid somatic conditions. Cardiovascular system diseases were recorded in 26.7% of patients (10/40), nervous

system disorders (excluding the primary psychiatric diagnosis) in 31.2% (12/40), and digestive system pathologies in 19.8% (8/40). Respiratory and skin conditions were present in lower frequencies.

3.1.2. Prevalence and Composition of Intestinal Parasitocenosis

The parasitological examination revealed that 32.5% (13/40; 95% CI: 19.5–48.7%) of the studied patients were infected with intestinal parasites. The distribution of parasitic infections is shown in Figure 1. Protozoan infections accounted for the majority of cases, detected in 27.5% of all patients (11/40; 95% CI: 15.1–43.9%), while helminth infection (Enterobius vermicularis) was found in 5% (2/40; 95% CI: 0.9–16.9%).

Blastocystis hominis was the predominant parasite, identified in 53.8% of all positive cases (7/13; 95% CI: 25.5–78.7%). Giardia lamblia was found in 30.8% (4/13; 95% CI: 11.0–60.6%), and Enterobius vermicularis in 15.4% (2/13; 95% CI: 2.7–46.3%). The species composition of the identified parasitocenosis is presented in Figure 2.

3.1.3. Antibiotic Susceptibility of Blastocystis hominis Isolates

All seven isolated *Blastocystis hominis* strains were tested for susceptibility to metronidazole and trimethoprim. Using the disk diffusion method, 42.9% of strains (3/7; 95% CI: 15.8–75.0%) were classified as sensitive to metronidazole, while the remaining 57.1% (4/7; 95% CI: 25.0–84.2%) were resistant. For trimethoprim, 57.1% of strains (4/7; 95% CI: 25.0–84.2%) were sensitive, and 42.9% (3/7; 95% CI: 15.8–75.0%) were resistant. McNemar's test revealed no statistically significant difference in the distribution of sensitive/resistant phenotypes between the two drugs within this cohort ($\chi^2 = 0.00$, $p = 1.00$), indicating a comparable, high level of resistance for both agents.

3.1.4. Minimum Inhibitory Concentration (MIC)

The quantitative broth microdilution assay confirmed the resistance patterns observed in the disk diffusion test. The determined MIC values were: 10^{-3} mg/mL for metronidazole and 10^{-4} mg/mL for trimethoprim. The sensitivity of the tested *Blastocystis hominis* strains to the study drugs is summarized in Figure 3.

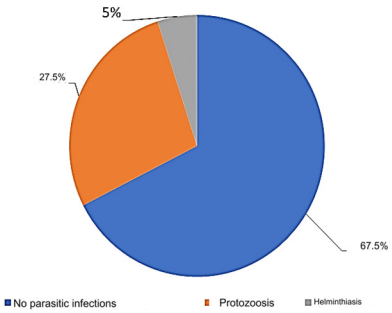

Figure 1. Distribution of intestinal parasitic infections among the examined patients with mental disorders (n=40). Data are presented as percentage of total patients with 95% confidence intervals.

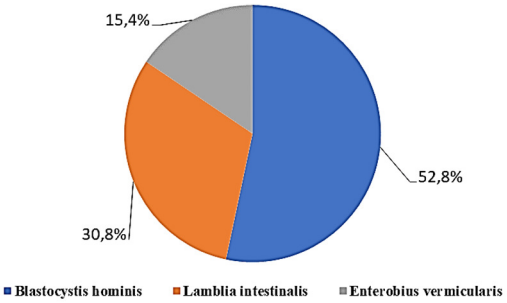

Figure 2. Species composition of intestinal parasitocenosis in infected patients (n=13). Data are presented as percentage of total positive cases.

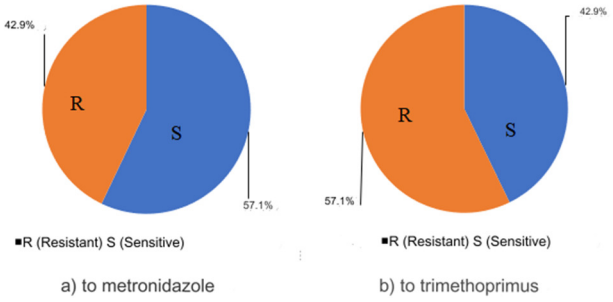

Figure 3. *In vitro* susceptibility of *Blastocystis hominis* strains (n=7) to metronidazole and trimethoprim. Left panel: Proportion of sensitive and resistant strains determined by disk diffusion method. Right panel: Determined Minimum Inhibitory Concentration (MIC) values for each drug.

3.2. Discussion

The present study provides novel and clinically significant insights into the intersection of antiprotozoal drug resistance, gut ecosystem dynamics, and mental healthcare. Our findings confirm the initial hypothesis that patients with mental and behavioral disorders—a cohort subject to long-term psychotropic pharmacotherapy—

represent a high-risk group not only for intestinal parasitic colonization but also for harboring drug-resistant strains of Blastocystis hominis. The data reveal a troubling epidemiological picture characterized by a high prevalence of parasitosis and substantial in vitro resistance to first-line therapeutic agents.

The overall prevalence of intestinal parasites (32.5%) in our cohort is markedly higher than rates typically reported for the general adult population in non-endemic regions of the Russian Federation. This elevated burden underscores specific vulnerabilities associated with severe mental illness. These may include factors related to the institutional environment, potential lapses in personal hygiene during acute phases of illness, and, most critically, the iatrogenic alteration of the intestinal microenvironment. Our finding that *Blastocystis hominis* was the dominant parasite (53.8% of infections) aligns with its global status as a common gut eukaryote. However, its predominance in this specific setting is noteworthy and may reflect a particular ecological advantage within a dysbiotic gut. This observation is consistent with recent findings by Franklin et al. (2022), who reported a higher prevalence of *Blastocystis* and metronidazole-resistant forms specifically in schizophrenic patients compared to controls.

The core and most alarming result of this investigation is the high level of resistance to metronidazole and trimethoprim observed in the isolated *Blastocystis hominis* strains. Only 42.9% and 57.1% of strains were sensitive to metronidazole and trimethoprim, respectively, with McNemar's test confirming no statistically significant difference in resistance profiles between the two drugs (p=1.00). This statistical equivalence indicates that within this cohort, the efficacy of both frontline agents is comparably compromised. The determined MIC values (10^{-3} mg/mL for metronidazole and 10^{-4} mg/mL for trimethoprim) quantitatively support this resistance, suggesting that supracinical concentrations would be required to achieve inhibition in vitro. These resistance rates exceed those described in some general population studies of Blastocystis susceptibility (Ilyina & Kasatkina, 2010; Kappagoda *et al*., 2011; Mirza et al., 2011; Roberts et al., 2015), which have demonstrated significant subtype-dependent variations and the existence of naturally less susceptible strains. However, the proportion of resistant isolates in our psychiatric cohort appears elevated compared to some general population surveys, pointing to a potential selection pressure unique to the psychiatric inpatient setting.

We posit that the long-term administration of antipsychotic medications is a key driver of this phenomenon, creating a perfect storm for the selection of resistant protozoa. As reviewed in the introduction, a growing body of evidence demonstrates that psychotropic drugs possess significant antimicrobial properties and can profoundly reshape the gut microbiota (Maier et al., 2018; Bugero, 2004). Drugs such as valproate and lithium have been shown to increase the abundance of Clostridium spp., while others alter microbial diversity and metabolic output. This drug-induced dysbiosis likely compromises the gut's natural colonization resistance—the ability of the resident microbiota to suppress pathogen expansion. Furthermore, an altered biochemical milieu (e.g., shifts in pH, redox potential, metabolite availability) may directly or indirectly apply selective pressure on parasitic populations. For instance, sub-inhibitory exposure to drug metabolites or a dysbiosis-associated inflammatory environment could enrich for protozoal strains with pre-existing or newly acquired resistance mechanisms. In related pathogens like Giardia lamblia, metronidazole resistance is linked to downregulation of nitroreductase enzymes (Kapoor *et al*., 2017; El-Taweel, 2015). It is plausible that the chronic, low-grade stress of a dysbiotic environment selects for Blastocystis subtypes or phenotypic variants with reduced drug activation or enhanced efflux capabilities. Our prior research supports this, showing a correlation between the persistent potential of Blastocystis spp. and the degree of intestinal dysbiosis (Bugero *et al*., 2020).

Our results find a compelling context within the broader literature on the gut-brain axis in psychiatry. Studies have consistently documented significant gut dysbiosis in patients with schizophrenia, depression, and bipolar disorder (Torshin *et al*., 2022; Li *et al*., 2021). While these studies focus on bacterial communities, they establish that the "psychiatric gut" is a distinct and altered ecosystem. Our work extends this concept by demonstrating that this dysbiotic state may also favor the establishment of drug-resistant eukaryotic parasites, adding a new layer of clinical complexity. The "microbiome psychopathogenicity" indices described by Torshin *et al*. (2022) may thus be indirectly linked to risks beyond metabolic and immune dysfunction, encompassing opportunistic parasitic infections that are difficult to treat.

The clinical implications of these findings are substantial. The standard empirical

prescription of metronidazole for suspected protozoal diarrhea in this patient population may be ineffective in over half of the cases involving Blastocystis hominis. Treatment failures can lead to chronic, unresolved gastrointestinal symptoms, which may be misattributed to functional disorders or side effects of psychotropic medication, leading to unnecessary diagnostic delays and altered psychiatric pharmacotherapy. Therefore, a paradigm shift in clinical practice is warranted. For psychiatric inpatients with persistent gastrointestinal symptoms, routine parasitological examination should be considered. More importantly, when *Blastocystis hominis* is detected, moving away from empirical therapy toward treatment guided by in vitro susceptibility testing, where feasible, could significantly improve outcomes. This study serves as a clear signal for the need for heightened vigilance regarding parasitic infections in mental health facilities.

4. CONCLUSIONS:

This study represents the first systematic investigation to identify and document the phenomenon of antiprotozoal drug resistance in *Blastocystis hominis* strains isolated from a vulnerable and pharmacologically unique cohort—patients with mental and behavioral disorders undergoing antipsychotic therapy. The key findings conclusively demonstrate:

A high prevalence of intestinal parasitosis (32.5%) was confirmed in the examined psychiatric inpatient cohort, with protozoan infections constituting the majority of cases (27.5%).

Blastocystis hominis was identified as the most prevalent intestinal parasite, accounting for over half (53.8%) of all positive findings.

A critically high level of resistance to first-line antiprotozoal drugs was revealed. Only 42.9% of *Blastocystis hominis* strains were sensitive to metronidazole, and 57.1% to trimethoprim, with no statistically significant difference in efficacy between the two drugs.

Quantitative MIC analysis confirmed the resistance, indicating the need for high drug concentrations to achieve inhibition in vitro.

The obtained results strongly suggest that the standard empirical prescription of antiprotozoal drugs, particularly metronidazole, in this patient population has a high probability of therapeutic failure. It is highly plausible that the long-term use of antipsychotic therapy, by inducing significant alterations in the gut microbiome (dysbiosis), creates an intestinal environment that not only facilitates colonization by opportunistic protozoa like *Blastocystis hominis* but also exerts a selective pressure favoring the survival and dominance of drug-resistant strains. Consequently, this research underscores an urgent necessity for a change in clinical practice: vigilant parasitological monitoring and a shift from empirical therapy towards treatment guided by antimicrobial susceptibility testing should be considered integral components of managing gastrointestinal comorbidities in patients with severe mental disorders.

5. DECLARATIONS

5.1. Study Limitations and Future Directions

The limitations of this study must be considered when interpreting its findings. The sample size, particularly of isolated strains (n=7), limits the statistical power and generalizability of the resistance rates, as reflected by the wide 95% confidence intervals. The retrospective, single-center design and the absence of a matched control group (e.g., non-psychiatric patients or healthy individuals from the same region) prevent definitive causal attribution of the observed resistance to psychotropic drugs. Methodologically, while adapted protocols and quality controls were employed, the lack of internationally standardized clinical breakpoints (e.g., CLSI/EUCAST) for Blastocystis hominis susceptibility testing remains a significant challenge for the field, making cross-study comparisons difficult. The use of disk diffusion criteria from prior literature (Sizenzov, 2009) is a necessary but imperfect solution. Furthermore, molecular subtyping (ST) of the Blastocystis isolates was not performed; given that different subtypes (ST1–ST4) may vary in pathogenic potential and drug susceptibility, this information would have added valuable depth to the analysis.

These limitations chart a clear course for future research. Large-scale, multi-center prospective studies are needed to validate the prevalence and resistance patterns observed here. Integrating molecular genotyping of Blastocystis isolates with phenotypic susceptibility testing is crucial to identify subtype–resistance associations. Developing and validating standardized, reproducible antimicrobial susceptibility testing protocols for intestinal protozoa remains an urgent methodological priority. Finally, experimental and longitudinal clinical studies are required to directly investigate the causal links between specific psychotropic

drugs, gut microbial and metabolic shifts, and the selection of drug-resistant parasitic strains. Understanding these mechanisms could lead to novel adjuvant strategies, such as probiotic or prebiotic interventions, aimed at restoring colonization resistance and reducing the burden of resistant infections in this vulnerable population.

5.2. Acknowledgments

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5.3. Funding Sources

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5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

The raw data supporting the findings of this study (anonymized patient data and primary susceptibility testing results) are available from the corresponding author (N.V.B.) upon reasonable request. The data are not publicly available due to restrictions pertaining to patient confidentiality and privacy, as per the regulations of the ethics committee that approved the study.

5.6. Author Contributions

Conceptualization and Design (CD): N.V.B., N.A.I. Data Collection (DC): A.A.T. Data Analysis and Interpretation (DAI): N.V.B., S.M.A., A.A.T. Manuscript Writing (MW): N.V.B., A.A.T. Critical Review (CR): S.M.A., A.I.K. Final Approval (FA): All authors. All authors have read and agreed to the published version of the manuscript.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors certify that this research adheres to the highest standards of research integrity. Specifically, we confirm: no data fabrication or falsification was performed; no p-hacking or selective reporting of results was conducted; this work is original and has not been published previously; all methods were conducted in accordance with relevant ethical guidelines and regulations.

5.9. Originality & Plagiarism Statement

This manuscript is the original work of the authors. The text was checked for plagiarism using the text.ru software. The overall similarity index was below 10%, confirming originality. All sources of literature are properly cited in APA format. This manuscript is not under consideration for publication elsewhere.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

The study protocol was reviewed and approved by the Independent Interdisciplinary Ethics Committee of Pskov State University (Protocol No. 245-EC/2022, dated April 15, 2022). The study was classified as a retrospective analysis of anonymized medical records and granted an exemption from full review, with expedited approval granted.

6.2. Informed Consent

For this retrospective study involving the analysis of pre-existing, fully anonymized medical records, the requirement for written informed consent was waived by the aforementioned Ethics Committee. All patient data were anonymized at the source institution prior to researcher access by removing all direct identifiers (name, address, ID number) and using a unique study code. Confidentiality and data privacy were protected in strict compliance with the Russian Federation's Federal Law № 152-FZ "On Personal Data" and the principles of the Declaration of Helsinki.

7. REFERENCES:

1. Abedi, S. H., Fazlzadeh, A., Mollalo, A., Sartip, B., Mahjour, S., Bahadory, S., Taghipour, A., & Rostami, A. (2022). The neglected role of *Blastocystis* sp. and *Giardia lamblia* in development of irritable bowel syndrome: A systematic review and meta-analysis. *Microbial Pathogenesis*, 162, 105215. https://doi.org/10.1016/j.micpath.2021.105215
2. Aykur, M., Karakavuk, E., Karakavuk, M., Akıl, M., Can, H., Döşkaya, M., Gürüz, Y., & Dağcı, H. (2020). Inhibitory effect of Tunceli garlic (*Allium tuncelianum*) on *Blastocystis* subtype 3 grown in vitro. *Expert Opinion on Orphan Drugs*, 8(11), 489–496. https://doi.org/10.1080/21678707.2020.1857725
3. Bremer Christensen, C., Soelberg, J., Stensvold, C. R., & Jäger, A. K. (2015). Activity of medicinal plants from Ghana against the parasitic gut protist *Blastocystis*. *Journal of Ethnopharmacology*, 174, 569–575. https://doi.org/10.1016/j.jep.2015.03.006
4. Bugero, N. V. (2004). *The role of microbial ecology in the pathogenesis of secondary infections in psychiatric patients* [Unpublished doctoral dissertation]. Pskov State University.
5. Bugero, N. V., Ilyina, N. A., & Alexandrova, S. M. (2020). Persistent potential of protozoa *Blastocystis* spp. *Periódico Tchê Química*, 17(35), 1057–1069. https://doi.org/10.52571/ptq.v17.n35.2020.87_bugero_pgs_1057_1069.pdf
6. Chernenkaya, T. V., & Godkov, M. A. (2015). "Problematic" multidrug-resistant bacteria - pathogens of hospital-acquired infections in patients in critical conditions (literature review). *N.V. Sklifosovsky Journal Urgent Medical Help*, 3, 30-35.
7. Courvalin, P. (2016). Why is antibiotic resistance a deadly emerging disease? *Clinical Microbiology and Infection*, 22, 405–407. https://doi.org/10.1016/j.cmi.2016.01.012
8. El-Taweel, H. A. (2015). Understanding drug resistance in human intestinal protozoa. *Parasitology Research*, 114(5), 1647–1659. https://doi.org/10.1007/s00436-015-4423-1
9. Franklin, F., Rajamanikam, A., Raju, C. S., Gill, J. S., Francis, B., Sy-Cherng, L. W., & Kumar, S. (2022). Higher amoebic and metronidazole resistant forms of *Blastocystis* sp. seen in schizophrenic patients. *Parasites & Vectors*, 15(1). https://doi.org/10.1186/s13071-022-05418-0
10. Gureser, A. S., Karasartova, D., Sarzhanov, F., Kosar, N., Taylan-Ozkan, A., & Dogruman-Al, F. (2023). Prevalence of *Blastocystis* and *Dientamoeba fragilis* in diarrheal patients in Corum, Türkiye. *Parasitology Research*, 122(12), 2977–2987. https://doi.org/10.1007/s00436-023-07987-0
11. Ilyina, N. A., & Kasatkina, N. M. (2010). *Blastocystis hominis* sensitivity to antiprotozoal drugs. *Bulletin of OSU*, 12, 38–

41.

12. Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3), 300–305. https://doi.org/10.4103/joacp.JOACP_349_15
13. Kappagoda, S., Singh, U., & Blackburn, B. G. (2011). Antiparasitic therapy. *Mayo Clinic Proceedings*, 86(6), 561–583. https://doi.org/10.4065/mcp.2011.0203
14. Li, S., Song, J., Ke, P., et al. (2021). The gut microbiome is associated with brain structure and function in schizophrenia. *Scientific Reports*, 11(1), 9743. https://doi.org/10.1038/s41598-021-89166-8
15. Maier, L., Pruteanu, M., Kuhn, M., Zeller, G., Telzerow, A., Anderson, E. E., Brochado, A. R., Fernandez, K. C., Dose, H., Mori, H., Patil, K. R., Bork, P., & Typas, A. (2018). Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature*, 555(7698), 623–628. https://doi.org/10.1038/nature25979
16. Meng, S. Q., Xue, Y. X., Xie, W., Shi, J., et al. (2021). Gut microbiota modulates the inflammatory response and cognitive impairment induced by sleep deprivation. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-021-01113-1
17. Mirza, H., Teo, J. D. W., Upcroft, J., & Tan, K. S. W. (2011). A rapid, high-throughput viability assay for *Blastocystis* spp. reveals metronidazole resistance and extensive subtype-dependent variations in drug susceptibilities. *Antimicrobial Agents and Chemotherapy*, 55(2), 637–648. https://doi.org/10.1128/aac.00900-10
18. Mukhina, E. G. (2017). Social problem of antibiotic resistance. *Universum: Medicine and Pharmacology*, 6(40). http://7universum.com/ru/med/archive/item/4898
19. Namazova-Baranova, L. S., & Baranov, A. A. (2017). Antibiotic resistance in the modern world. *Pediatric Pharmacology*, 14(5), 341–354. https://doi.org/10.15690/pf.v14i5.1782
20. Nurtayeva, K. S., Dzhumasheva, R. T., & A.Zh. (2018). Current state of the role of protozoa and parasitic worms in human parasitocenosis. *Bulletin of the Kazakh National Medical University*, 1, 476–478.
21. Roberts, T., Bush, S., Ellis, J., Harkness, J., & Stark, D. (2015). In vitro antimicrobial susceptibility patterns of *Blastocystis*. *Antimicrobial Agents and Chemotherapy*, 59(8), 4417–4423. https://doi.org/10.1128/aac.04832-14
22. Rudzińska, M., & Sikorska, K. (2023). Epidemiology of *Blastocystis* infection: A review of data from Poland in relation to other reports. *Pathogens*, 12(8), 1050. https://doi.org/10.3390/pathogens12081050
23. Rudzki, L., Stone, T. W., Maes, M., Misiak, B., Samochowiec, J., & Szulc, A. (2021). Gut microbiota-derived vitamins - underrated powers of a multipotent ally in psychiatric health and disease. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 107, 110240. https://doi.org/10.1016/j.pnpbp.2020.110240
24. Stensvold, C. R., Smith, H. V., Nagel, R., Olsen, K. E. P., & Traub, R. J. (2010). Eradication of *Blastocystis* carriage with antimicrobials: Reality or delusion? *Journal of Clinical Gastroenterology*, 44(2), 85–90. https://doi.org/10.1097/mcg.0b013e3181bb86ba
25. Sizenzov, A. N. (2009). *Methods for determining antibiotic productivity and antibiotic resistance: guidelines for laboratory practical training*. Orenburg State University.
26. Torshin, I. Yu., Gromova, O. A., Vanchakova, N. P., & Semenov, V. A. (2022). Comparative analysis of microbiome parameters in patients with mental disorders and healthy volunteers. *Experimental and Clinical Gastroenterology*, 204(8), 93-95.
27. Wang, Z., Chen, W. H., Li, S. X., et al. (2021). Oral microbiota changes in elderly patients, an indicator of Alzheimer's disease. *International Journal of Environmental Research and Public Health*, 18(8), 4211. https://doi.org/10.3390/ijerph18084211

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

NESFATINA-1 COMO BIOINDICADOR DE DESREGULAÇÃO METABÓLICA EM DOENÇAS GASTROINTESTINAIS: ANÁLISE DE UM ESTUDO CASO-CONTROLE

NESFATIN-1 AS A BIOINDICATOR OF METABOLIC DYSREGULATION IN GASTROINTESTINAL DISEASES: ANALYSIS OF A CASE–CONTROL STUDY

النيسفاتين-1 كمؤشر حيوي لإختلال التنظيم الأيضي في أمراض الجهاز الهضمي :تحليل دراسة الحالات-الشواهد

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RESUMO

Introdução: A Nesfatina-1 (Nes-1), um peptídeo derivado da nucleobindina-2 (NUCB2), tem sido cada vez mais reconhecida por seu papel na regulação do apetite, do metabolismo da glicose e do equilíbrio lipídico. Evidências crescentes indicam que alterações nos níveis de Nesfatina-1 também podem estar associadas a distúrbios metabólicos relacionados a doenças gastrointestinais (GI). **Objetivo:** Este estudo teve como objetivo investigar os níveis séricos de Nesfatina-1 em pacientes com distúrbios gastrointestinais e avaliar suas associações com parâmetros metabólicos e seu potencial valor diagnóstico. **Métodos:** Estudo de caso-controle conduzido com 128 participantes: 88 pacientes com doenças gastrointestinais e 40 controles aparentemente saudáveis. Os participantes foram recrutados após exame endoscópico no Centro Especializado em Gastroenterologia e Hepatologia, Al-Najaf Al-Ashraf, Iraque, no período de 1º de setembro a 25 de dezembro de 2025. Foram realizadas avaliações laboratoriais incluindo Nesfatina-1 sérica, parâmetros do perfil lipídico (colesterol total, LDL, HDL e triglicerídeos), glicemia, hemoglobina e enzimas hepáticas (ALT e AST). O índice aterogênico do plasma (IAP) foi calculado. As análises estatísticas incluíram estatística descritiva, modelagem de regressão e análise da curva ROC. **Resultados:** Os níveis séricos de Nesfatina-1 foram significativamente menores nos pacientes com doenças gastrointestinais em comparação aos controles saudáveis (11,82 ± 2,19 vs. 20,20 ± 7,14 ng/mL, $p < 0,001$). A análise demonstrou que, à medida que os níveis de Nesfatina-1 diminuíam, o IMC, a glicose, o colesterol total, o LDL, os triglicerídeos e a razão TG/HDL aumentavam. A análise ROC demonstrou elevada eficácia diagnóstica do teste, com área sob a curva (AUC) de 0,91, sendo o melhor ponto de corte de 13,68 ng/mL, com sensibilidade de 80,7% e especificidade de 85,0%. **Discussão:** Esses achados sugerem que a redução dos níveis de Nesfatina-1 pode refletir uma desregulação metabólica subjacente nas doenças gastrointestinais. **Conclusão:** A Nesfatina-1 pode representar um biomarcador promissor para a predição de distúrbios metabólicos e para apoio à avaliação clínica dos distúrbios gastrointestinais.

Palavras-chave: *Nesfatina-1; Doenças gastrointestinais; distúrbios metabólicos; perfil lipídico; índice aterogênico.*

ABSTRACT

Background: Nesfatin-1 (Nes-1), a peptide derived from nucleobindin-2 (NUCB2), has been increasingly recognized for its role in regulating appetite, glucose metabolism, and lipid balance. Growing evidence indicates that alterations in Nesfatin-1 levels may also be linked to metabolic disturbances associated with gastrointestinal (GI) diseases. **Aim**: This study aimed to investigate serum Nesfatin-1 levels in patients with gastrointestinal disorders and to evaluate their associations with metabolic parameters and potential diagnostic value. **Methods**:

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A case-control study was conducted with 128 participants: 88 patients with gastrointestinal diseases and 40 apparently healthy controls. Participants were recruited after endoscopic examination at the Gastroenterology and Hepatology Specialized Center in Al-Najaf Al-Ashraf, Iraq, from September 1 to December 25, 2025. It performed laboratory assessments, including serum Nesfatin-1, lipid profile parameters (total cholesterol, LDL, HDL, and triglycerides), blood glucose, hemoglobin, and liver enzymes (ALT and AST). The atherogenic index of plasma (AIP) was calculated. Statistical analyses included descriptive statistics, regression modeling, and receiver operating characteristic (ROC) curve analysis. **Results**: Serum Nesfatin-1 levels were markedly lower in patients with gastrointestinal diseases than in healthy controls (11.82 ± 2.19 vs. 20.20 ± 7.14 ng/mL, $p < 0.001$). Analysis showed that as Nesfatin-1 levels decreased, BMI, glucose, total cholesterol, LDL, triglycerides, and the TG/HDL ratio increased, ROC analysis showed that the test was very effective at diagnosing, with an area under the curve (AUC) of 0.91, and the best cutoff point was 13.68 ng/mL, achieving a sensitivity of 80.7% and a specificity of 85.0%. **Discussion**: These findings suggest that reduced Nesfatin-1 levels may reflect underlying metabolic dysregulation in gastrointestinal diseases. **Conclusion**: Nesfatin-1 may represent a promising biomarker for predicting metabolic disturbances and supporting the clinical evaluation of gastrointestinal disorders.

Keywords: *Nesfatin-1; Gastrointestinal diseases; metabolic disturbances; lipid profile; and atherogenic index.*

الملخص

الخلفية: يُعدّ النيسفاتين-1(Nes-1)، وهو ببتيد مشتق من النيوكليوبيندين-2 (NUCB2)، من الجزيئات التي حظيت باهتمام متزايد لدوره في تنظيم الشهية واستقلاب الغلوكوز وتوازن الدهون. وتشير الأدلة المتزايدة إلى أن التغيرات في مستويات نيسفاتين-1 قد ترتبط أيضًا بالاضطرابات الأيضية المصاحبة لأمراض الجهاز الهضمي. **الهدف**: هدفت هذه الدراسة إلى تقصّي مستويات نيسفاتين-1 في مصل الدم لدى المرضى المصابين باضطرابات الجهاز الهضمي، وتقييم علاقتها بالمعلمات الأيضية وقيمتها التشخيصية المحتملة. **طرائق العمل**: أُجريت دراسة حالة–شاهد شملت 128 مشاركًا: 88 مريضًا يعانون من أمراض الجهاز الهضمي و40 شخصًا سليمًا ظاهريًا كمجموعة ضابطة. وتم اختيار المشاركين بعد إجراء الفحص التنظيري في مركز أمراض الجهاز الهضمي والكبد التخصصي في النجف الأشرف، العراق، خلال الفترة من 1 أيلول إلى 25 كانون الأول 2025. وقد أُجريت تحاليل مخبرية شملت قياس نيسفاتين-1 في المصل، ومعلمات الملف الدهني (الكوليسترول الكلي، LDL، HDL، والدهون الثلاثية)، وسكر الدم، والهيموغلوبين، وإنزيمات الكبد (ALT وAST). كما تم حساب مؤشر التصلب العصيدي في البلازما (AIP). وشملت التحليلات الإحصائية الإحصاء الوصفي، ونمذجة الانحدار، وتحليل منحنى الخصائص التشغيلية للمستقبِل (ROC).**النتائج**: كانت مستويات نيسفاتين-1 في المصل أقل بشكل ملحوظ لدى المرضى المصابين بأمراض الجهاز الهضمي مقارنةً بالأشخاص الأصحاء (11.82 ± 2.19 مقابل 20.20 ± 7.14 نانوغرام/مل، $p < 0.001$). وأظهر التحليل أنه مع انخفاض مستويات نيسفاتين-1 ترتفع قيم مؤشر كتلة الجسم (BMI)، والغلوكوز، والكوليسترول الكلي، وLDL، والدهون الثلاثية، ونسبة TG/HDL. كما أظهر تحليل ROC أن الاختبار يتمتع بفعالية عالية في التشخيص، حيث بلغت المساحة تحت المنحنى (AUC) 0.91، وكانت أفضل نقطة قطع 13.68 نانوغرام/مل، مع حساسية بلغت 80.7% ونوعية بلغت 85.0%. **المناقشة**: تشير هذه النتائج إلى أن انخفاض مستويات نيسفاتين-1 قد يعكس وجود خلل في التنظيم الأيضي لدى مرضى الجهاز الهضمي. **الاستنتاج**: قد يمثّل نيسفاتين-1 مؤشرًا حيويًا واعدًا للتنبؤ بالاضطرابات الأيضية ودعم التقييم السريري لاضطرابات الجهاز الهضمي.

الكلمات المفتاحية: النيسفاتين-1؛ أمراض الجهاز الهضمي؛ الاضطرابات الأيضية؛ الملف الدهني؛ المؤشر التصلبي الوعائي.

1. INTRODUCTION:

Gastrointestinal (GI) diseases are a significant global health issue, frequently associated with metabolic disorders that may influence disease progression and treatment efficacy. In recent years, there has been growing interest in regulatory peptides that link the physiology of the gastrointestinal system to the body's overall metabolic balance. Nesfatin-1 (Nes-1) is a bioactive peptide that comes from nucleobindin-2 (NUCB2). It is now an important regulator of metabolic function and energy balance (Schalla & Stengel, 2019).

Researchers first identified nesfatin-1 in the hypothalamus, where it helps regulate hunger and energy levels. Later research showed that this peptide is present throughout the body, not just in the central nervous system. It can also be found in the pancreas, stomach, fat tissue, and intestinal mucosa (Stengel & Taché, 2021). Nesfatin-1 is found in many different tissues, suggesting it does more than control hunger. It is very important for controlling metabolism and how the digestive system works.

There is growing evidence that nesfatin-1 helps stabilize blood sugar levels and aid fat breakdown. Nesfatin-1 can alter insulin secretion, glucose utilization, and lipid breakdown. This suggests it may be involved in metabolic disorders such as obesity, insulin resistance, and dyslipidemia (Aydin, 2021; Schalla *et al*., 2021). Changes in circulating nesfatin-1 levels have also been linked to several metabolic disorders. This suggests that this peptide may be a sign of metabolic imbalance (Algul *et al*., 2020).

New studies show that nesfatin-1 may also affect the digestive system, not just metabolism. Previous studies have shown that nesfatin-1 can alter gastric motility, the body's response to inflammation, and communication

between the gut and brain (Li *et al*., 2022). These results suggest that nesfatin-1 may be involved in the complicated link between metabolic control and gastrointestinal disease.

There is increasing evidence about how nesfatin-1 works in the body, but few clinical studies have examined its effects on gastrointestinal diseases. It's not at all clear how changes in metabolism in people with GI disorders are linked to blood nesfatin-1 levels. Learning more about this link could reveal how metabolic processes work in people with gastrointestinal diseases and help us identify new biomarkers for diagnosis.

1.1. Aims

Consequently, the present study aimed to evaluate serum nesfatin-1 concentrations in patients with gastrointestinal disorders and to investigate their association with metabolic parameters, including lipid profiles and glucose levels. This study also sought to examine how well nesfatin-1 could distinguish between patients with gastrointestinal disorders and healthy controls.

2. MATERIALS AND METHODS:

2.1. Material

2.1.1. Study Design and Population

This case–control study was performed from September to December 2025 at Gastroenterology and Hepatology Specialized Center in Al-Najaf Al-Ashraf, Iraq. The study included 128 participants aged 15 to 70. All participants underwent upper gastrointestinal endoscopy, a procedure that involves inserting a flexible endoscope with a camera through the mouth to visualize the esophagus, stomach, and duodenum for diagnostic purposes. Based on clinical and endoscopic findings, participants were divided into two groups: 88 patients with gastrointestinal diseases and 40 apparently healthy individuals who constituted the control group. Demographic and clinical data were collected through standardized questionnaires and medical record review. These data encompassed age, sex, body mass index (BMI), smoking status, and pertinent clinical history. Participants with a history of chronic liver disease, renal disease, autoimmune disorders, metabolic diseases, malignancy, prior gastric surgery, or those undergoing medications that could affect metabolic parameters were excluded from the study.

The sample size was determined based on the availability of qualified participants during the study period and in alignment with analogous previously published research on nesfatin-1 levels in clinical populations.

2.2. Methods

2.2.1. Body Mass Index (BMI)

The computation of BMI is performed by a balance and height device, for calculating the weight and height, and applying Equation 1, to measure body mass index (BMI), calculated as weight in kilograms divided by the square of height in meters (kg/m^2) (Feng *et al*., 2019).

BMI = weight (kg) / Height (m^2) (Eq.1)

2.2.2. Blood Sample Collection

Under sterile conditions, about 5 mL of venous blood was taken from each participant. The blood samples were allowed to clot and then spun at 3000 rpm for 10 minutes to separate the serum. We kept the serum samples at −20 °C until we could perform additional biochemical tests.

2.2.3. Determination of Serum Nesfatin-1

Serum Nesfatin-1 (Nes-1) concentrations were determined using a commercial Human Nesfatin-1 ELISA kit supplied by BT LAB, China (Cat. No. E3063Hu) according to the manufacturer's instructions. The assay is based on the sandwich enzyme-linked immunosorbent assay (ELISA) technique. Microplate wells are pre-coated with antibodies specific for human Nesfatin-1. Serum samples are added to the wells, allowing Nesfatin-1 present in the samples to bind to the immobilized antibodies. A biotin-labeled anti-Nesfatin-1 antibody is then added, followed by streptavidin-horseradish peroxidase (HRP). After incubation and washing steps, the substrate solution is added, resulting in a color reaction proportional to the concentration of Nesfatin-1 in the sample.

The assay has a standard curve range of 0.3–90 ng/mL and a sensitivity of 0.15 ng/mL. According to the manufacturer, the assay demonstrated acceptable precision with an intra-

assay coefficient of variation (CV) < 8% and inter-assay CV < 10%.

2.2.4. Biochemical Measurements

Biochemical parameters were analyzed using the Cobas c111 clinical chemistry analyzer (Roche Diagnostics). The following parameters were measured: Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein cholesterol (HDL-C), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST).

Specific Roche diagnostic reagents were used, including ALT (Cat. No. 20764911) and AST (Cat. No. 20764920). Quality control was performed using PreciControl ClinChem Multi 1 and Multi 2 together with Calibrator f.a.s. (Roche Diagnostics) to ensure analytical accuracy and reliability. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated using the Friedewald equation (Equations 2 and 3). Lipid concentrations were initially calculated in mg/dL and subsequently converted to mmol/L for the lipid profile.

[Analyte] (mg/dl) = (Absorbance of Sample/ Absorbance of Standard) ×[Standard] (mg/dl (Eq.2)

[LDL-C] (mg/dl) = [Total Cholesterol] - ([Triglycerides] / 5) - [HDL Cholesterol]; [VLDL-C] (mg/dl) = [Triglycerides] / 5 (Eq.3)

2.2.5. Random Blood Glucose Measurements

Random blood glucose (RBG) levels were measured in serum samples using an automated biochemical analyzer (Cobas c111, Roche Diagnostics), according to the manufacturer's standard procedures. The method is based on the enzymatic colorimetric glucose oxidase technique, which allows quantitative determination of glucose concentration in blood samples. Quality control procedures were performed regularly using standard calibration materials to ensure the accuracy and reliability of the measurements.

2.2.6. Calculation of Atherogenic Index of Plasma

The atherogenic index of plasma (AIP) was calculated to evaluate the atherogenic risk associated with lipid metabolism. AIP was determined using the logarithmic transformation of the ratio between triglycerides and high-density lipoprotein cholesterol according to Equation 4.

AIP = log10 (Triglycerides / HDL-C) (Eq.4)

where triglycerides and HDL-C are expressed in mmol/L.

2.2.7. Investigation of Helicobacter pylori

The assessment of Helicobacter pylori (H. pylori) infection was conducted using two diagnostic techniques: the urea breath test and the stool antigen test, each selected to provide complementary diagnostic accuracy. In the urea breath test, the Heliprobe® system (manufactured in Sweden) was used to detect H. pylori infection by measuring urease activity. Participants were instructed to fast for 6 to 7 hours prior to the procedure to ensure optimal test sensitivity. Initially, a baseline breath sample was obtained by having the patient exhale into a balloon-like container or tube. Subsequently, the patient ingested a urea solution labeled with a carbon isotope, either radioactive or non-radioactive, designed to react with urease produced by H. pylori. After a 10–30-minute wait, a second breath sample was collected. The two breath samples were then analyzed and compared to detect the presence of labeled carbon dioxide, indicative of urease-mediated urea hydrolysis by H. pylori. This stepwise approach enabled precise detection of H. pylori infection with high sensitivity and specificity.

For the stool antigen test, stool samples were collected and analyzed by an enzymatic immunoassay using a commercial diagnostic kit specifically developed for H. pylori antigen detection. The enzyme immunoassay utilized monoclonal antibodies to identify H. pylori antigens, providing a non-invasive diagnostic alternative that complements the urea breath test. The combined use of both diagnostic methods was intended to enhance overall diagnostic accuracy and mitigate the risk of false-negative results, thereby ensuring a more comprehensive assessment of H. pylori infection status.

2.3. Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 27. Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as

frequencies and percentages when appropriate. The normality of the data distribution was evaluated using Q–Q plots. Differences between study groups were analyzed using appropriate statistical tests for quantitative variables. The relationship between serum Nesfatin-1 and metabolic parameters was assessed using multiple linear regression analysis.

Furthermore, receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of serum Nesfatin-1 in distinguishing patients with gastrointestinal diseases from healthy controls. The area under the curve (AUC), sensitivity, specificity, and optimal cutoff value were determined. A p-value < 0.05 was considered statistically significant.

The effect size for the primary outcome (serum Nesfatin-1) was calculated using Cohen's d. The observed effect size was large (Cohen's d = 1.92), indicating that the sample was adequate to detect the between-group difference with high precision

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Baseline Characteristics of the Study Population

The statistical analysis compares GI patients to healthy controls in Table 1. There was no significant difference in age (36.81 ± 13.88 vs. 35.38 ± 10.78 years, p = 0.564), age groups (>35, 25–34, 15–24; p = 0.602), or sex distribution (p = 0.088). GI patients had a higher BMI (26.25 ± 3.45 vs. 24.90 ± 2.95, p = 0.026) and a higher proportion were classified as overweight or obese. Fasting glucose levels were higher in GI patients (106.62 ± 21.43 vs. 91.38 ± 9.66 mg/dL, p < 0.001), while hemoglobin levels were lower (11.75 ± 1.57 vs. 12.71 ± 1.16 g/dL, p < 0.001), and there were more cases of moderate and mild anemia (p = 0.052). In GI patients, liver enzymes ALT and AST were slightly lower (ALT: 12.68 ± 6.06 vs. 14.91 ± 5.35 U/L, p = 0.040; AST: 16.92 ± 5.20 vs. 18.97 ± 5.45 U/L, p = 0.049). However, total serum bilirubin did not differ significantly (p = 0.376). A lipid profile analysis showed that GI patients had higher triglycerides (2.13 ± 0.58 vs. 1.42 ± 0.37 mmol/L, p < 0.001) and TG/HDL ratios (1.87 ± 0.76 vs. 1.06 ± 0.38, p < 0.001) but lower HDL (1.21 ± 0.20 vs. 1.37 ± 0.18 mmol/L, p < 0.001). Total cholesterol and LDL levels were not significantly different. The atherogenic index of plasma (AIP) showed that more GI patients were at moderate to high risk (39.8% vs. 7.5%, p < 0.001). Nes-1 levels were markedly diminished in GI patients (11.82 ± 2.19 vs. 20.20 ± 7.14, p < 0.001). Regarding medication, 39.8% of GI patients were receiving treatment for their primary diagnosis or biological therapy, whereas none of the controls received any medication (p = 0.037).

3.1.2. Distribution and comparative concentrations of serum Nesfatin-1

Figure 1 shows the distribution of serum Nesfatin-1 (Nes-1) levels and their comparison between patients with gastrointestinal (GI) diseases and healthy controls.

The Q–Q plots in Figures 1A and 1B show the distribution of Nes-1 values across the two study groups. This is how we check for normality. In patients with GI (Figure 1A), the data points are very close to the reference diagonal line. This shows that the observed values are mostly in line with a normal distribution, with only small differences at the extreme values. The control group (Figure 1B) also shows a roughly linear pattern along the reference line, with small changes at the lower and upper tails of the distribution. In general, the plots show that the Nes-1 values in both groups are distributed close to normal.

Figure 1C shows that the mean serum Nes-1 level in patients with gastrointestinal diseases was 11.82 ± 2.19 ng/mL. In contrast, the control group had a higher mean value of 20.20 ± 7.14 ng/mL. Statistical analysis showed that the difference between the two groups was very significant (P < 0.001).

3.1.3. Distribution of Subdiagnosis of Gastrointestinal Disease and Associated Nesfatin-1 Levels

The results in Figure 2 show how the different types of gastrointestinal (GI) diseases are distributed among patients and how much Nesfatin-1 (Nes-1) is in their blood. Organic gastrointestinal disorders accounted for the majority of cases (69 patients, 78%), while functional disorders accounted for 19 patients (22%), indicating a statistically significant predominance of organic conditions ($\chi^2 = 28.41$, P < 0.001), as shown in Figure 2A.

Figure 2B's statistical analysis showed a very big difference in Nes-1 levels between the study groups (P < 0.001). The primary factor

contributing to this difference was the significantly elevated Nes-1 levels in the healthy control group compared to all gastrointestinal disease categories. In contrast, the patient groups displayed relatively uniform concentrations within a narrow range (approximately 10–12 ng/mL).

Irritable bowel syndrome (IBS) was the most common diagnosis, accounting for 22% of cases (n = 19). IBS patients, despite being the most common diagnosis, showed a moderate drop in Nes-1 levels, with a mean concentration of 12.17 ± 2.22 ng/mL compared to the healthy control group (20.20 ± 7.14 ng/mL).

The second most common condition was Helicobacter pylori–associated gastritis (20%, n = 18), with patients having a mean Nes-1 level of 12.22 ± 2.19 ng/mL, indicating that their circulating Nes-1 levels were similar to those of healthy people. Ulcerative colitis, which accounted for 19% of the cases (n = 17), had a mean concentration of 11.95 ± 2.29 ng/mL, slightly lower than that of the other cases.

Crohn's disease, comprising 13% of the cohort (n = 11), had the lowest mean Nes-1 level among all gastrointestinal disease groups (10.08 ± 1.46 ng/mL), suggesting a greater reduction in circulating Nes-1 in this inflammatory bowel disease subtype. Conversely, celiac disease, representing 11% of the patient cohort (n = 10), exhibited a mean Nes-1 level of 12.39 ± 2.34 ng/mL, which is consistent with levels found in other non-ulcerative gastrointestinal disorders.

Gastric ulcer (6%, n = 5) and GERD with gastritis (6%, n = 5) were less common, with mean Nes-1 concentrations of 10.31 ± 1.23 ng/mL and 11.38 ± 1.03 ng/mL, respectively. Small intestinal ulcer, constituting the smallest proportion of cases (3%, n = 3), exhibited a comparatively elevated mean Nes-1 level (14.03 ± 2.50 ng/mL) relative to other gastrointestinal diseases.

3.1.3. Linear Regression Analysis of Factors Associated with Nesfatin-1 Levels

The current study used multiple linear regression to examine the association between serum Nesfatin-1 (Nes-1) levels and various clinical and biochemical factors in individuals with gastrointestinal diseases (Table 2). Figure 4's scatterplot shows the overall regression relationship between standardized predicted values and observed Nes-1 concentrations.

The study found that body mass index (BMI) was strongly and negatively linked to Nes-1 levels (B = −0.26, β = −0.41, p = 0.005). This means that people with higher BMI tended to have lower levels of Nes-1 in their blood.

There was a very strong negative relationship between fasting glucose and Nes-1 (B = −0.07, β = −0.73, p < 0.001). This suggests that higher glucose levels are strongly linked to lower Nes-1 levels.

Similarly, several lipid profile parameters showed significant negative correlations with Nes-1. These included total cholesterol (TC) (B = −0.90, β = −0.37, p < 0.001), low-density lipoprotein cholesterol (LDL-C) (B = −1.01, β = −0.40, p < 0.001), triglycerides (TG) (B = −0.90, β = −0.24, p = 0.024), and the TG/HDL ratio (B = −0.75, β = −0.26, p = 0.013). These results show that higher levels of atherogenic lipids are linked to lower levels of Nes-1.

On the other hand, high-density lipoprotein cholesterol (HDL-C) was strongly associated with Nes-1 (B = 3.04, β = 0.28, p = 0.009), suggesting that higher HDL levels may be associated with higher Nes-1 levels in the blood.

However, there were no statistically significant links between age (p = 0.282), hemoglobin (p = 0.086), ALT (p = 0.660), AST (p = 0.972), or total serum bilirubin (p = 0.385) and Nes-1 levels.

Figure 3 shows a scatterplot of the linear relationship between standardized predicted values and serum Nesfatin-1 (Nes-1) levels in patients with gastrointestinal diseases. Each point represents a different patient. The fitted linear model shown by the red regression line is y = 11.82 + 1.74x. This indicates a positive linear relationship between the predicted regression values and the measured Nes-1 levels.

3.1.4. Diagnostic Performance of Nesfatin-1 Based on ROC Curve Analysis

Receiver operating characteristic (ROC) curve analysis (Figure 4) was conducted to assess the diagnostic performance of serum Nesfatin-1 (Nes-1) in differentiating patients with gastrointestinal (GI) diseases from healthy controls. The analysis demonstrated that Nes-1 possesses excellent discriminatory capacity, with an area under the curve (AUC) of 0.91 (95% confidence interval: 0.85–0.96, p < 0.001).

The optimal cutoff value for Nes-1 was determined to be 13.68 ng/mL, based on the

maximum Kolmogorov–Smirnov statistic, which identifies the point of greatest separation between patient and control distributions. At this threshold, Nes-1 achieved a sensitivity of 80.7% and a specificity of 85.0%.

3.2. Discussion

The current study demonstrates that patients with gastrointestinal (GI) diseases exhibited significantly reduced circulating nesfatin-1 levels compared to healthy controls, accompanied by a metabolic profile characterized by elevated BMI, increased fasting glucose, heightened triglycerides, an elevated TG/HDL ratio, and diminished HDL levels. When taken together, these results point to a gut–metabolic axis rather than a local intestinal event. This interpretation is biologically plausible because nesfatin-1 is not only a central anorexigenic peptide but is also expressed in peripheral tissues, including the gastric mucosa, pancreas, adipose tissue, and other components of the digestive system. Recent reviews consistently characterize nesfatin-1 as a multifunctional regulator of appetite, glucose metabolism, lipid metabolism, oxidative stress, and inflammatory signaling, rendering it pertinent to heterogeneous gastrointestinal cohorts, including the one examined in this study (Damian-Buda *et al*., 2024; Dore *et al*., 2017; Gonkowski *et al*., 2022; Schalla & Stengel, 2018; Stengel & Taché, 2011).

From a mechanistic standpoint, the regression results align with the experimental literature. Nesfatin-1 was initially characterized as a satiety-related NUCB2-derived peptide in the hypothalamus (Oh-I *et al*., 2006); subsequent research, however, has demonstrated that its effects extend significantly beyond appetite regulation. Experimental studies have shown that nesfatin-1 can directly increase glucose-dependent insulin secretion in β-cells, make insulin more effective, change how the liver makes glucose, and change how insulin-target tissues take up glucose (Gonzalez *et al*., 2011; Li *et al*., 2013; Wu *et al*., 2014; Geng *et al*., 2025). This establishes a robust biological foundation for the observation that reduced nesfatin-1 levels correlate with elevated fasting glucose levels and less favorable metabolic indices. Instead of being a single peptide change, lower levels of nesfatin-1 in the GI group may be a sign of problems in a larger endocrine network that connects nutrient sensing, intestinal signaling, insulin response, and liver metabolism (Geng *et al*., 2025).

The current lipid results are just as important. The negative correlations between nesfatin-1 and total cholesterol, LDL, triglycerides, and the TG/HDL ratio, along with the positive correlation with HDL, align with the increasing body of literature linking nesfatin-1 to lipid metabolism and cardiometabolic risk. Reviews and mechanistic studies demonstrate that nesfatin-1 is involved in hepatic lipid regulation, adipocyte biology, and systemic lipid homeostasis (Luo *et al*., 2021; Dore *et al*., 2017). This indicates that the diminished nesfatin-1 observed in GI patients may not solely reflect disease burden; it may also delineate a subgroup exhibiting a more atherogenic metabolic phenotype. AIP findings, along with the fact that the GI patients in the cohort were more metabolically unfavorable than controls, even though the age and sex distributions were similar, support that interpretation. In terms of the thesis, this is a strong point: the peptide is not only lower in disease, but it also has a biologically coherent metabolic profile that goes along with it.

Simultaneously, the clinical literature regarding circulating nesfatin-1 is not entirely consistent; some studies have found that nesfatin-1 levels were lower in people with higher BMI or metabolic problems. For example, in non-obese men, there was an inverse relationship with BMI, and in overweight patients, there was a predictive relationship with metabolic syndrome (Tsuchiya *et al*., 2010; Alsarraf *et al*., 2025). Other research has reported elevated levels of circulating nesfatin-1 in newly diagnosed type 2 diabetes or in specific obesity-related contexts (Zhang *et al*., 2012; Ramanjaneya *et al*., 2010). This variability indicates that serum nesfatin-1 is context-dependent and may fluctuate based on adiposity compartment, disease stage, medication status, inflammatory tone, assay platform, and whether the cohort is primarily metabolic, inflammatory, or mixed. The most defensible conclusion for this dataset is not that nesfatin-1 is present in every disorder, but that in this heterogeneous GI population, it correlated with an adverse metabolic state and distinctly differentiated patients from controls.

Another significant aspect of the present findings is that nesfatin-1 levels were diminished across gastrointestinal subdiagnoses, exhibiting only minor variations among the disease categories. This pattern contradicts a strictly disease-specific interpretation and supports the notion that nesfatin-1 signifies a common pathophysiological disturbance among gastrointestinal disorders. Recent reviews of

digestive diseases support that view by placing nesfatin-1 at the crossroads of inflammatory signaling, oxidative balance, mucosal regulation, and metabolic control (Damian-Buda *et al*., 2024). Studies indicating the involvement of nesfatin-1 in gut physiology, gastric function, and brain–gut stress signaling provide further support (Gonkowski *et al*., 2022; Yang *et al*., 2017; Stengel & Taché, 2011). Consequently, the uniformity of reduction across GI subgroups may suggest that the prevailing signal is not “Crohn’s vs ulcer vs IBS,” but rather “GI disease with systemic metabolic strain” versus health.

These findings regarding peptic ulcers are also relevant in a broader clinical context. A recent review in JAMA confirms that peptic ulcer disease is still strongly linked to H. pylori, NSAID exposure, and clinically important complications. The additional Iraqi study suggests that ulcer patients may have both biochemical and metabolic problems simultaneously (Vakil, 2024; Abd-Alameer & Sharba, 2025). This does not demonstrate that dyslipidemia or altered nesfatin-1 induces ulcer disease; however, it substantiates the overarching assertion that gastrointestinal disorders frequently coexist with systemic biochemical disturbances. Nesfatin-1 may function less as a specific “GI lesion marker” and more as a biomarker of the metabolic-inflammatory environment associated with GI disease.

Lastly, the ROC analysis demonstrated a robust discriminatory capacity of serum Nesfatin-1 in differentiating gastrointestinal patients from healthy controls (AUC = 0.91). This discovery indicates that Nesfatin-1 could serve as a potential biomarker linked to metabolic changes in gastrointestinal disorders. However, as the results were derived from a single case–control dataset, additional validation in larger, more heterogeneous populations is necessary before affirming its clinical applicability.

4. CONCLUSIONS:

The current study found that patients with gastrointestinal diseases had significantly lower serum Nesfatin-1 levels than healthy controls. The distribution analysis showed that Nesfatin-1 values were roughly normally distributed in both groups. This supports the accuracy of the statistical comparisons. The ROC curve analysis also showed that Nesfatin-1 was very effective at distinguishing healthy individuals from those with gastrointestinal problems, with an area under the curve (AUC) of 0.91. The cutoff value of 13.68 ng/mL showed good sensitivity (80.7%) and specificity (85.0%), indicating it was effective at distinguishing between the two groups.

In general, these results indicate that people with gastrointestinal disorders have much lower serum Nesfatin-1 levels. This could be a useful biomarker for distinguishing between sick and healthy people.

5. DECLARATIONS

5.1. Study Limitations

The study had several limitations that should be considered when interpreting the findings. This study has several limitations that should be acknowledged. First, the study was conducted at a single medical center, which may limit the generalizability of the findings to broader populations. Second, although the sample size was determined based on participant availability during the study period, the observed effect size for the primary outcome was large (Cohen's d = 1.92), suggesting that the sample was sufficient to detect the main between-group difference. Nevertheless, a formal a priori sample size calculation was not performed, and future studies should include pre-specified power calculations to strengthen the methodological rigor. Third, the case–control design limits the ability to establish causal relationships between Nesfatin-1 levels and metabolic alterations in gastrointestinal diseases. Additionally, the study evaluated serum Nesfatin-1 at a single time point, which may not fully reflect potential dynamic changes over time. Future studies involving larger multicenter cohorts and longitudinal designs are recommended to further validate these findings and clarify the role of Nesfatin-1 in gastrointestinal disease–related metabolic dysregulation.

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5.4. Competing Interests

The authors declare that they have no competing interests or conflicts of interest that could have influenced the work presented in this manuscript. This includes, but is not limited to, financial relationships, personal affiliations, intellectual property considerations, or other potential sources of bias. All authors have reviewed and approved this declaration, ensuring transparency and maintaining scientific integrity in the reporting of this research.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author.

5.6. Author Contributions

Swar Adnan Mohammed (SAM): Collected data (DC), contributed to manuscript writing (MW), and approved the final version of the manuscript (FA). Intisar Razzaq Sharba (IRS): Led the conception and design of the study (CD), performed data analysis and interpretation (DAI), contributed to manuscript writing (MW), conducted critical review (CR), and approved the final version of the manuscript (FA). Jinan Mohammed Zahid (JMZ): Contributed to the conception and design of the study (CD), participated in manuscript writing (MW), conducted critical review (CR), and approved the final version of the manuscript (FA).

5.7. AI and Computational Tools Declaration

The authors declare that artificial intelligence (AI) tools were used solely for minor language editing to improve grammar, sentence structure, and overall clarity of the manuscript. AI was not used in the design of the study, data collection, statistical analysis, interpretation of the results, or in generating the scientific conclusions. All scientific content, analyses, and interpretations presented in this work are the responsibility of the authors.

5.8. Research Integrity Declaration

The authors confirm that this research complies with accepted standards of research integrity. The study involved no data fabrication, no results falsification, and no p-hacking or selective reporting. The work is original, has not been previously published, and all methods were conducted ethically.

5.9. Originality & Plagiarism Statement

The authors confirm that this manuscript is original work and has not been previously published or submitted elsewhere for publication. No text has been copied from other sources without proper citation, and all references have been appropriately cited in APA format. The manuscript is not currently under consideration by any other journal.

5.10. Open Access

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki for research involving human

participants. Ethical and academic approval was obtained from the Council of the University of Kufa, College of Science, under official letter No. 5373 dated October 1, 2025. In addition, administrative and research approval to conduct the study in healthcare facilities was granted by the Najaf Health Directorate, Ministry of Health, Iraq, through the Training and Human Development Center, under official letter No. 27321 dated October 7, 2025. The approval permitted conducting the study at the Specialized Hospital for Gastrointestinal and Liver Diseases in Al-Najaf Al-Ashraf, Iraq.

All participants were informed of the study's purpose and procedures prior to enrollment, and written informed consent was obtained from each participant. The confidentiality of personal information was strictly maintained, and all collected data and biological samples were used exclusively for scientific research purposes..

6.2. Informed Consent

The participants involved in the current study were patients seeking diagnosis and treatment at the Gastroenterology and Hepatology Specialized Center in Al-Najaf Al-Ashraf, Iraq, for standard diagnostic evaluations. Biological specimens and relevant data were collected following defined laboratory protocols. Verbal informed consent was obtained from all adult participants, whereas consent for minors was collected from a parent or legal guardian. Participants were informed of the study's aims and their right to withdraw from participation at any moment. All gathered data were anonymized and assigned unique identifiers prior to analysis, with access rigorously restricted to the research team to preserve anonymity and comply with ethical standards.

7. REFERENCES:

1. Algul, S., Ozkan, Y., & Ozcelik, O. (2020). Serum nesfatin-1 levels and metabolic risk factors. Endocrine Research, 45(3), 203–210. https://doi.org/10.1080/07435800.2020.1719971
2. Aydin, S. (2021). Nesfatin-1 and its role in metabolic regulation: An updated review. Peptides, 136, 170424. https://doi.org/10.1016/j.peptides.2020.170424
3. Li, Z., Gao, L., Tang, H., Yin, Y., Xiang, X., & Li, Y. (2022). The role of nesfatin-1 in gastrointestinal function and metabolic regulation. Frontiers in Endocrinology, 13, 865432. https://doi.org/10.3389/fendo.2022.865432
4. Schalla, M. A., & Stengel, A. (2019). Current understanding of nesfatin-1. Peptides, 119, 170104. https://doi.org/10.1016/j.peptides.2019.170104
5. Schalla, M. A., Prinz, P., Friedrich, T., & Stengel, A. (2021). Nesfatin-1 and metabolic diseases: Current perspectives. Frontiers in Endocrinology, 12, 660164. https://doi.org/10.3389/fendo.2021.660164
6. Stengel, A., & Taché, Y. (2021). Nesfatin-1: A metabolic and gastrointestinal regulatory peptide. Peptides, 135, 170424. https://doi.org/10.1016/j.peptides.2020.170424
7. Abd-Alameer, Z. M., & Sharba, I. R. (2025). Metabolic dysregulation and risk predictors of peptic ulcer disease: A clinical and biochemical analysis. Zenodo.
8. Alsarraf, Z., Mohammed, M. H., & Haitham, F. (2025). The role of serum nesfatin-1 in predicting metabolic syndrome among patients with overweight. Regulatory Mechanisms in Biosystems, 16(3), e25123. doi:10.15421/0225123
9. Damian-Buda, A.-C., Matei, D. M., Ciobanu, L., Damian-Buda, D.-Z., Pop, R. M., Buzoianu, A. D., & Bocsan, I. C. (2024). Nesfatin-1: A novel diagnostic and prognostic biomarker in digestive diseases. Biomedicines, 12(8), 1913. doi:10.3390/biomedicines12081913
10. Dore, R., Levata, L., Lehnert, H., & Schulz, C. (2017). Nesfatin-1: Functions and physiology of a novel regulatory peptide. Journal of Endocrinology, 232(1), R45-R65. doi:10.1530/JOE-16-0361
11. Dotania, K., Tripathy, M., & Rai, U. (2021). A comparative account of nesfatin-1 in vertebrates. General and Comparative Endocrinology, 313, 113874. doi:10.1016/j.ygcen.2021.113874
12. Geng, S., Yang, S., Tang, X., Xue, S., Li, K., Liu, D., Chen, C., Zhu, Z., Zheng, H., Wang, Y., Yang, G., Li, L., & Yang, M. (2025). Intestinal NUCB2/nesfatin-1 regulates hepatic glucose production via the MC4R-cAMP-GLP-1 pathway. EMBO

Journal, 44, 54-74. doi:10.1038/s44318-024-00300-4
13. Gonkowski, S., Rychlik, A., & Całka, J. (2022). Peripheral nesfatin-1 in regulation of the gastrointestinal tract. Animals, 12(1), 101. doi:10.3390/ani12010101
14. Gonzalez, R., Reingold, B. K., Gao, X., Gaidhu, M. P., Tsushima, R. G., & Unniappan, S. (2011). Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet beta- and MIN6 cells. Journal of Endocrinology, 208(3), R9-R16. doi:10.1530/JOE-10-0492
15. Li, Z., Gao, L., Tang, H., Yin, Y., Xiang, X., Li, Y., Zhao, J., Mulholland, M., & Zhang, W. (2013). Peripheral effects of nesfatin-1 on glucose homeostasis. PLoS ONE, 8(8), e71513. doi:10.1371/journal.pone.0071513
16. Li, Q. C., Wang, H. Y., Chen, X., Guan, H. Z., & Jiang, Z. Y. (2010). Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. Regulatory Peptides, 159(1-3), 72-77.
17. Luo, J., Wen, F.-J., Qiu, D., Wang, S.-Z., Li, Y., Peng, Y., & Yang, G. (2021). Nesfatin-1 in lipid metabolism and lipid-related diseases. Clinica Chimica Acta, 522, 23-30. doi:10.1016/j.cca.2021.08.005
18. Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., Hashimoto, K., Tsuchiya, T., Monden, T., Horiguchi, K., Yamada, M., & Mori, M. (2006). Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature, 443, 709-712. doi:10.1038/nature05162
19. Pan, W., Hsuchou, H., & Kastin, A. J. (2007). Nesfatin-1 crosses the blood-brain barrier without saturation. Peptides, 28(11), 2223-2228. doi:10.1016/j.peptides.2007.09.005
20. Price, C. J., Samson, W. K., & Ferguson, A. V. (2008). Nesfatin-1 inhibits NPY neurons in the arcuate nucleus. Brain Research, 1230, 99-106. doi:10.1016/j.brainres.2008.06.084
21. Prinz, P., & Stengel, A. (2016). Expression and regulation of peripheral NUCB2/nesfatin-1. Current Opinion in Pharmacology, 31, 25-30. doi:10.1016/j.coph.2016.08.012
22. Ramanjaneya, M., Chen, J., Brown, J. E., Tripathi, G., Hallschmid, M., Patel, S., Kern, W., Hillhouse, E. W., Lehnert, H., Tan, B. K., & Randeva, H. S. (2010). Identification of nesfatin-1 in human and murine adipose tissue: A novel depot-specific adipokine with increased levels in obesity. Endocrinology, 151(7), 3169-3180. doi:10.1210/en.2009-1358
23. Schalla, M. A., & Stengel, A. (2018). Current understanding of the role of nesfatin-1. Journal of the Endocrine Society, 2(10), 1188-1206. doi:10.1210/js.2018-00246
24. Stengel, A., Goebel, M., Wang, L., & Taché, Y. (2011). Minireview: Nesfatin-1—An emerging new player in the brain-gut, endocrine, and metabolic axis. Endocrinology, 152(11), 4033-4038. doi:10.1210/en.2011-1500
25. Stengel, A., & Taché, Y. (2010). Nesfatin-1—Role as possible new potent regulator of food intake. Regulatory Peptides, 163(1-3), 18-23.
26. Tsuchiya, T., Shimizu, H., Yamada, M., Osaki, A., Oh-I, S., Ariyama, Y., Takahashi, H., Okada, S., Hashimoto, K., Satoh, T., Kojima, M., & Mori, M. (2010). Fasting concentrations of nesfatin-1 are negatively correlated with body mass index in non-obese males. Clinical Endocrinology, 73(4), 484-490. doi:10.1111/j.1365-2265.2010.03835.x
27. Vakil, N. (2024). Peptic ulcer disease: A review. JAMA, 332(21), 1832-1842. doi:10.1001/jama.2024.19094
28. Wu, D., Yang, M., Chen, Y., Jia, Y., Ma, Z., Boden, G., Li, L., & Yang, G. (2014). Hypothalamic nesfatin-1/NUCB2 knockdown augments hepatic gluconeogenesis that is correlated with inhibition of mTOR-STAT3 signaling pathway in rats. Diabetes, 63(4), 1234-1247.
29. Xu, Y., & Chen, F. (2020). Antioxidant, anti-inflammatory and anti-apoptotic activities of nesfatin-1: A review. Journal of Inflammation Research, 13, 607-617. doi:10.2147/JIR.S273446
30. Yang, G.-T., Zhao, H.-Y., Kong, Y., Sun, N.-N., & Dong, A.-Q. (2017). Study of the effects of nesfatin-1 on gastric function in obese rats. World Journal of Gastroenterology, 23(16), 2940-2947. doi:10.3748/wjg.v23.i16.2940
31. Zhang, Z., Li, L., Yang, M., Liu, H., Boden, G., & Yang, G. (2012). Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes, 120(2), 91-95. doi:10.1055/s-0031-1286339

Table 1. *Demographic, Clinical, and Biochemical Characteristics of GI Patients and Healthy Controls*

Variable	GI patients N=88	Controls N=40	95% CI	p-value
Age (yr.)	36.81 ± 13.88	35.38 ± 10.78	-3.05 – 5.91	0.564
>35	44 (50.0%)	19 (47.5%)		0.602 χ^2= 1.02
25–34	26 (29.5%)	15 (37.5%)		
15–24	18 (20.5%)	6 (15.0%)		
Sex				
Male	26 (29.5%)	18 (45.0%)		χ^2= 2.912 0.088
Female	62 (70.5%)	22 (55.0%)		
BMI	26.25 ± 3.45	24.90 ± 2.95	0.16 – 2.53	0.026*
Obese	13 (14.8%)	5 (12.5%)		0.256 χ^2= 2.73
Overweight	38 (43.2%)	12 (30.0%)		
Normal weight	37 (42.0%)	23 (57.5%)		
Glucose (mg/dL)	106.62 ± 21.43	91.38 ± 9.66	9.80 – 20.67	<0.001*
Hb (g/dL)	11.75 ± 1.57	12.71 ± 1.16	-1.46 – -0.47	<0.001*
Anemia				
Moderate	12 (13.6%)	3 (7.5%)		0.052 χ^2= 5.90
Mild	30 (34.1%)	7 (17.5%)		
Normal	46 (52.3%)	30 (75.0%)		
ALT (U/L)	12.68 ± 6.06	14.91 ± 5.35	-4.34 – -0.11	0.040*
AST (U/L)	16.92 ± 5.20	18.97 ± 5.45	-4.09 – -0.01	0.049*
T.S.B (mg/dL)	0.35 ± 0.10	0.37 ± 0.11	-0.06 – 0.02	0.376
TC (mmol/L)	4.11 ± 0.89	4.20 ± 0.71	-0.39 – 0.20	0.525
LDL (mmol/L)	1.92 ± 0.86	2.17 ± 0.74	-0.54 – 0.05	0.105
TG (mmol/L)	2.13 ± 0.58	1.42 ± 0.37	0.54 – 0.88	<0.001*
HDL (mmol/L)	1.21 ± 0.20	1.37 ± 0.18	-0.23 – -0.09	<0.001*
TG/HDL	1.87 ± 0.76	1.06 ± 0.38	0.61 – 1.01	<0.001*
AIP Class				
Moderate to High (>2)	35 (39.8%)	3 (7.5%)		<0.001* χ^2= 13.72
Normal (<2)	53 (60.2%)	37 (92.5%)		
Nes-1	11.82 ± 2.19	20.20 ± 7.14	-10.71 – -6.06	<0.001*
Medication				
Primary diagnosis	35 (39.8%)	0 (0.0%)		0.037* χ^2= 6.57
Yes	35 (39.8%)	0 (0.0%)		
Biology	18 (20.5%)	0 (0.0%)		

Significant differences at *p<0.05. Continuous variables as Mean ± SD and 95% CI. Categorical variables as N (%), χ^2, and p-value. AIP: Atherogenic index plasma

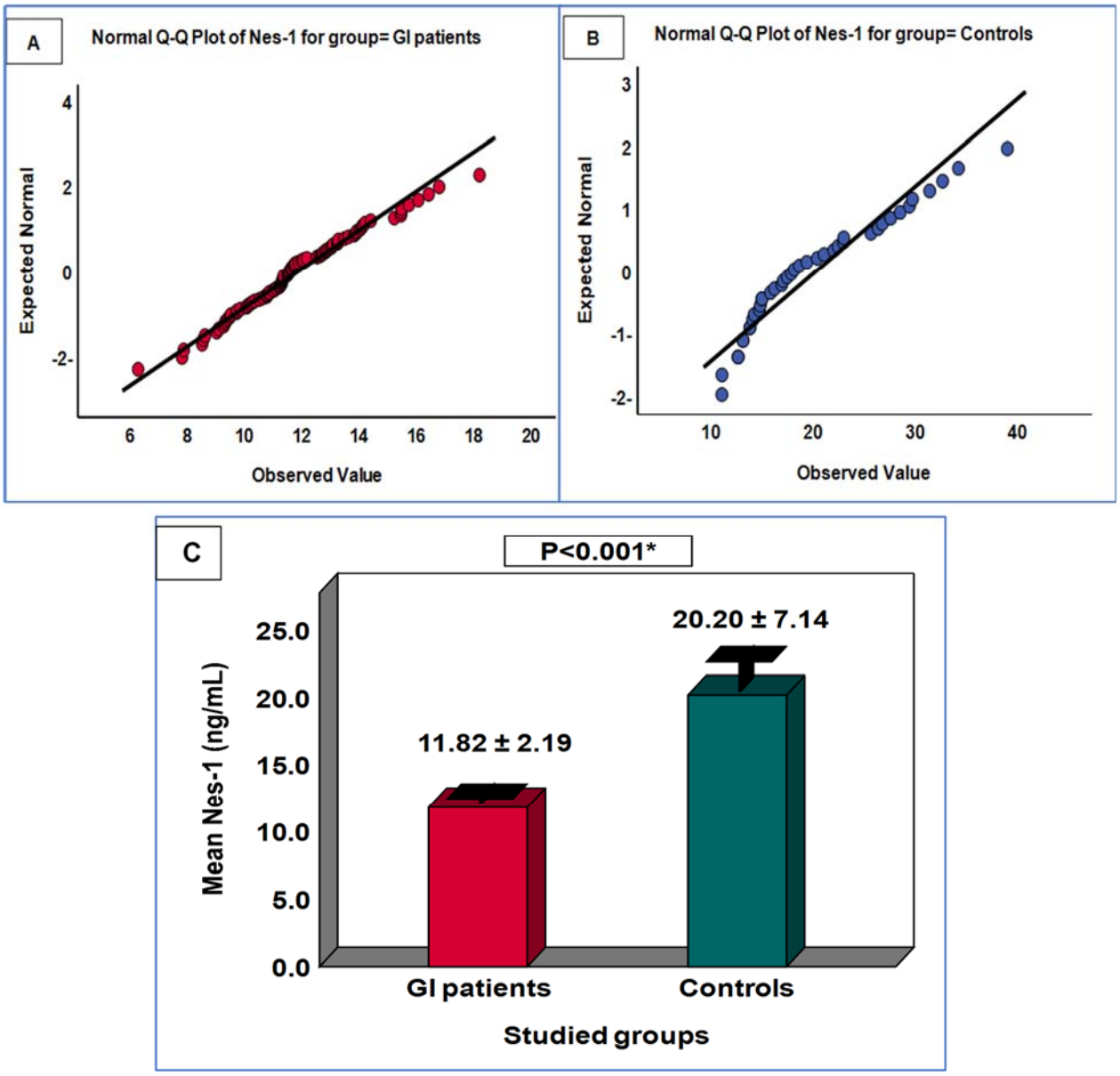

Figure 1. *The Q–Q plots of the Normal Distribution of Nes-1 in the GI and the Control groups*

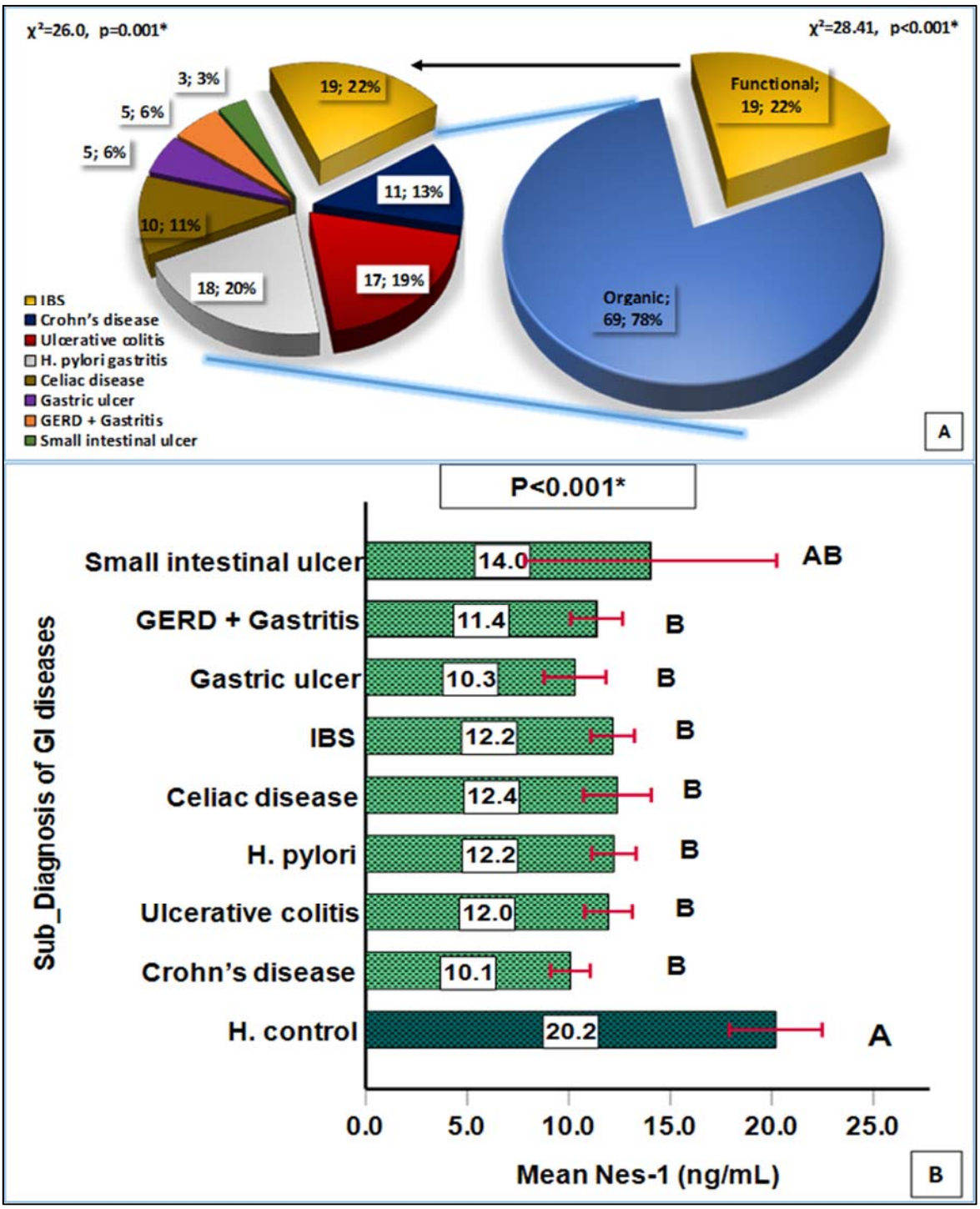

Figure 2. *Distribution of gastrointestinal disease, subdiagnosis, and serum Nesfatin-1 levels among patients and healthy controls*

Table 2. *Univariate linear regression analysis of clinical and biochemical predictors of serum Nesfatin-1 levels in gastrointestinal disease patients*.

Variable	B	Beta	t	p-value	95% CI
Age (yr.)	0.02	0.15	1.08	0.282	-0.02 – 0.07
BMI	-0.26	-0.41	-2.92	0.005**	-0.44 – -0.08
Glucose (mg/dL)	-0.07	-0.73	-9.84	<0.001***	-0.09 – -0.06
Hb (g/dL)	0.26	0.18	1.74	0.086	-0.04 – 0.55
ALT (U/L)	0.02	0.05	0.44	0.660	-0.06 – 0.09
AST (U/L)	-0.00	-0.00	-0.04	0.972	-0.09 – 0.09
T.S.B (mg/dL)	-2.14	-0.09	-0.87	0.385	-7.01 – 2.73
TC (mmol/L)	-0.90	-0.37	-3.66	<0.001***	-1.39 – -0.41
LDL (mmol/L)	-1.01	-0.40	-4.03	<0.001***	-1.51 – -0.51
TG (mmol/L)	-0.90	-0.24	-2.30	0.024*	-1.69 – -0.12
HDL (mmol/L)	3.04	0.28	2.69	0.009**	0.79 – 5.29
TG/HDL	-0.75	-0.26	-2.53	0.013*	-1.35 – -0.16

Dependent variable: Nes-1 (ng/mL). Significant differences at *p<0.05, **<0.01, ***<0.001

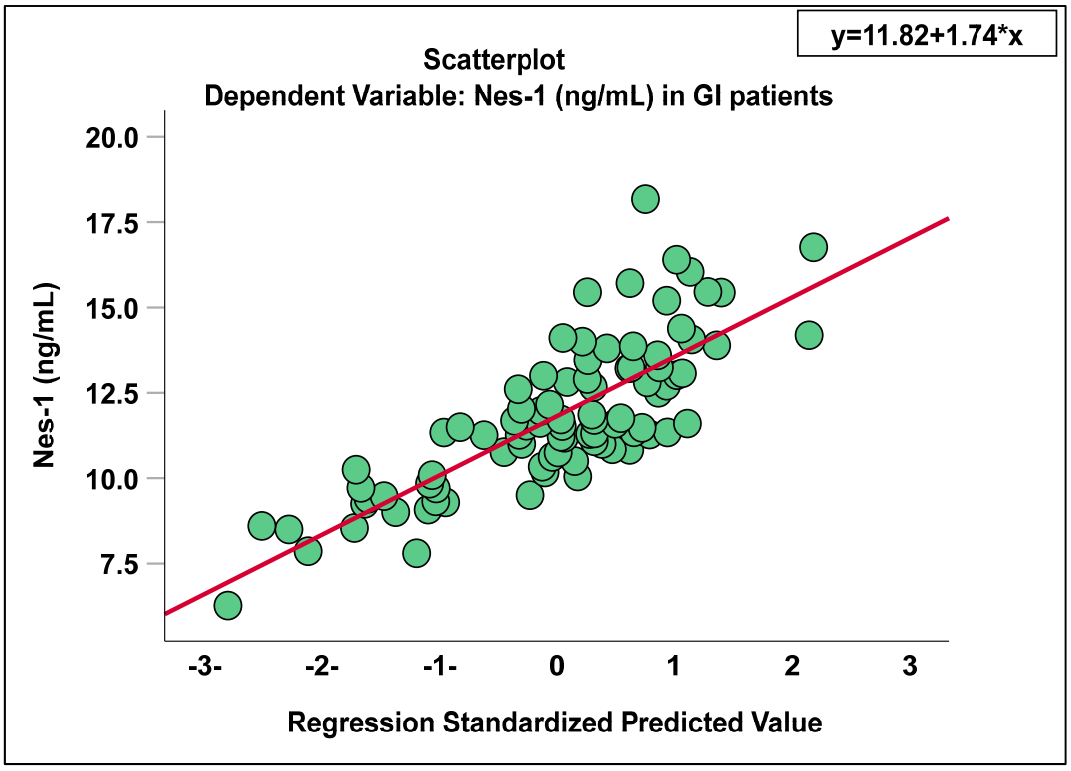

Figure 3. *Linear regression scatterplot of the relationship between standardized predicted values and serum Nesfatin-1 levels in patients with gastrointestinal diseases.*

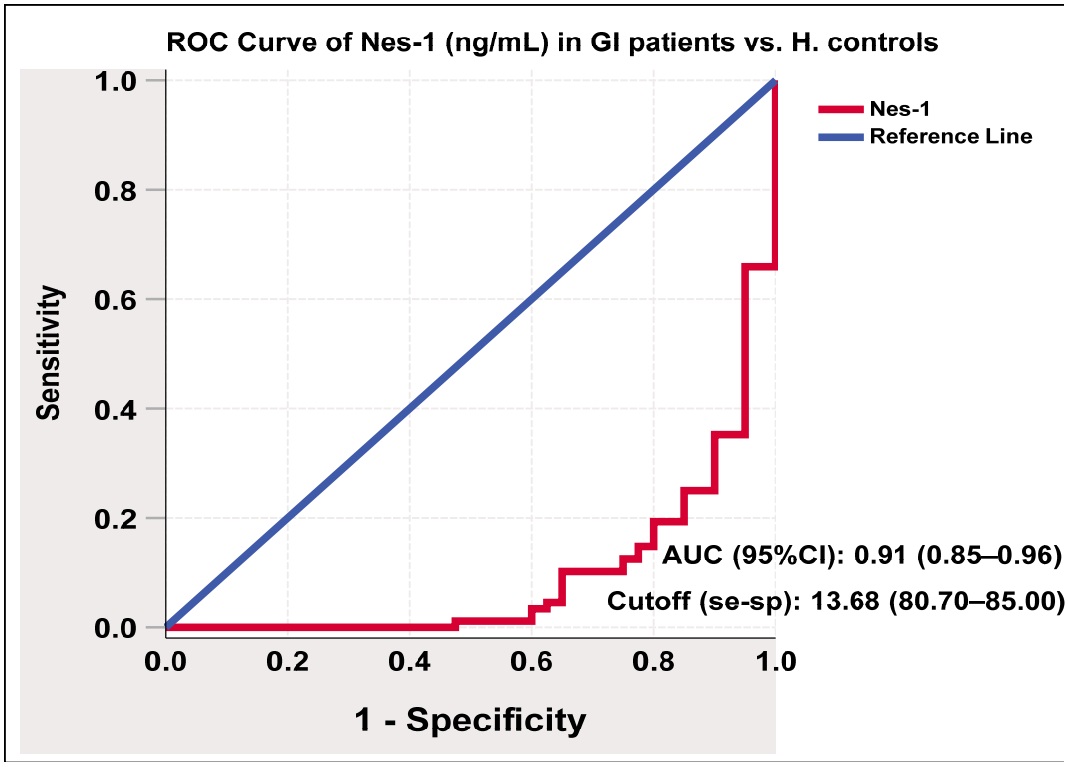

Figure 4. *ROC Curve Analysis of Serum Nesfatin-1 in GI Patients and Healthy Controls*

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

INVESTIGAÇÃO DO DESEMPENHO TÉRMICO-EXERGÉTICO DE UM AQUECEDOR SOLAR DE AR DE BAIXO CUSTO CONSTRUÍDO COM MATERIAIS LOCALMENTE DISPONÍVEIS

A THERMAL –EXERGY PERFORMANCE INVESTIGATION OF A COST-EFFECTIVE SOLAR AIR HEATER CONSTRUCTED FROM LOCALLY AVAILABLE MATERIALS

تحليل الأداء الحراري والإكسرجي لسخان هواء شمسي منخفض التكلفة مصنع من مواد متوفرة محلياً

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RESUMO

Introdução: Os aquecedores solares de ar (ASAs) representam uma tecnologia solar passiva promissora para aplicações de aquecimento de ambientes e secagem. Apesar de seu potencial, a combinação da avaliação de desempenho térmico (primeira lei) e exergético (segunda lei) de ASAs fabricados com materiais de baixo custo e localmente disponíveis ainda é pouco explorada. **Objetivo**: Este estudo teve como objetivo projetar, construir e avaliar experimentalmente três configurações de absorvedores de baixo custo para ASAs. **Métodos**: Três configurações de absorvedores (tubo de alumínio, mangueira corrugada de folha de alumínio e placa trapezoidal de aço galvanizado) foram construídas na Universidade de Misan, Maysan, Iraque. O ASA foi testado sob convecção natural e forçada, com ângulos de inclinação de 25°, 30° e 35°. A eficiência térmica foi calculada utilizando a primeira lei da termodinâmica, enquanto a eficiência exergética foi determinada por meio do modelo do fator de Petela. **Resultados**: Sob convecção natural, o absorvedor trapezoidal de aço alcançou o maior incremento de temperatura do ar (~65 °C próximo ao meio-dia solar). O ângulo de inclinação ótimo foi de 30°, proporcionando uma temperatura do ar na saída de aproximadamente 90 °C. Sob convecção forçada, a eficiência térmica atingiu um máximo de 22,9% e a eficiência exergética alcançou 0,14% para o absorvedor trapezoidal, ambos superiores aos das configurações de mangueira de folha de alumínio (21%; 0,13%) e tubo de alumínio (20%; 0,12%). O custo total de fabricação variou entre USD 120 e 150. **Discussão**: A convecção forçada melhorou a transferência de calor ao aumentar o coeficiente convectivo e reduzir a resistência da camada limite térmica, resultando em eficiências superiores tanto pela primeira quanto pela segunda lei da termodinâmica, apesar de um menor incremento de temperatura. As baixas eficiências exergéticas são consistentes com a literatura para sistemas solares térmicos de baixa temperatura e refletem a irreversibilidade inerente à conversão de radiação solar de alta qualidade em calor de baixa qualidade. **Conclusões**: O absorvedor trapezoidal de aço galvanizado, orientado a 30° e operado sob convecção forçada moderada, constitui a configuração de ASA mais eficiente do ponto de vista termodinâmico e mais econômica entre as avaliadas. Os resultados sustentam a viabilidade de ASAs fabricados localmente para aplicações domésticas e agrícolas em regiões rurais e de alta irradiância.

Palavras-chave: *Aquecedor Solar de Ar; Eficiência exergética; Eficiência térmica; Materiais de baixo custo; Absorvedor trapezoidal.*

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ABSTRACT

Background: Solar air heaters (SAHs) represent a promising passive solar technology for space heating and drying applications. Despite their potential, the combination of thermal (first-law) and exergy (second-law) performance evaluation for SAHs fabricated from low-cost, locally available materials remains largely unexplored. **Aim:** This study aimed to design, build, and experimentally evaluate three low-cost SAH absorber configurations. **Methods:** Three absorber configurations (i.e., aluminium tube, corrugated aluminium foil hose, and trapezoidal galvanized steel plate) were constructed at the University of Misan, Maysan, Iraq. The SAH was tested under natural and forced convections at tilt angles of 25°, 30°, and 35°. Thermal efficiency was calculated using the first law of thermodynamics, while exergy efficiency was determined using the Petela factor model. **Results:** Under natural convection, the trapezoidal steel absorber achieved the highest air temperature increment (~65 °C near solar noon). The optimal tilt angle was 30°, yielding an outlet air temperature of approximately 90°C. Under forced convection, the trapezoidal absorber achieved thermal efficiency of 22.9% and exergy efficiency of 0.14%, both superior to the aluminium foil hose (21%; 0.13%) and aluminium tube (20%; 0.12%) configurations. Total fabrication cost ranged from USD 120 to 150. **Discussion:** Forced convection improved heat transfer by increasing the convective coefficient and reducing thermal boundary layer resistance, resulting in superior first- and second-law efficiencies despite a lower temperature increment. The low exergy efficiencies are consistent with the literature for low-temperature solar thermal systems and reflect the inherent irreversibility of converting high-grade solar radiation into low-grade heat. **Conclusions:** The trapezoidal galvanized steel absorber, oriented at 30° and operated under moderate forced convection, constitutes the most thermodynamically efficient and cost-effective SAH configuration among those evaluated. The findings support the viability of locally manufactured SAHs for domestic and agricultural applications in rural and high-irradiance regions.

Keywords: *Solar Air Heater; Exergy efficiency; Thermal efficiency; Low-cost materials; Trapezoidal absorber.*

الملخص

الخلفية: تُعدّ سخانات الهواء الشمسية (SAHs) إحدى التقنيات الشمسية السلبية الواعدة في تطبيقات التدفئة وتجفيف المواد. وعلى الرغم من الإمكانات الكبيرة لهذه الأنظمة، فإن الدراسات التي تجمع بين تقييم الأداء الحراري (القانون الأول للديناميكا الحرارية) والأداء الإكسرجي (القانون الثاني) لسخانات الهواء الشمسية المصنَّعة من مواد منخفضة التكلفة ومتوفرة محلياً ما تزال محدودة. **الهدف:** هدفت هذه الدراسة إلى تصميم وتصنيع وتقييم الأداء التجريبي لثلاثة نماذج منخفضة التكلفة لممتصات سخان الهواء الشمسي. **طرائق العمل:** تم تصنيع ثلاث نماذج من الممتصات (أنبوب من الألمنيوم، خرطوم مموج من رقائق الألمنيوم، وصفيحة فولاذية مجلفنة ذات شكل شبه منحرف) في جامعة ميسان، محافظة ميسان، العراق. وتم اختبار سخان الهواء الشمسي في حالتي الحمل الطبيعي والقسري عند زوايا ميل قدرها 25° و30° و35°. حُسبت الكفاءة الحرارية باستخدام القانون الأول للديناميكا الحرارية، بينما حُددت الكفاءة الإكسرجية باستخدام نموذج عامل بيتلا (Petela). **النتائج:** في حالة الحمل الطبيعي، حقق الممتص الفولاذي شبه المنحرف أعلى زيادة في درجة حرارة الهواء (حوالي 65 °م عند ذروة الإشعاع الشمسي). وكانت زاوية الميل المثلى 30°، حيث بلغت درجة حرارة الهواء الخارج نحو 90 °م. أمّا في حالة الحمل القسري، فقد حقق الممتص شبه المنحرف كفاءة حرارية بلغت 22.9% وكفاءة إكسرجية بلغت 0.14%، وهي قيم أعلى من تلك التي حققها خرطوم رقائق الألمنيوم (21%؛ 0.13%) وأنبوب الألمنيوم (20%؛ 0.12%). وتراوحت تكلفة التصنيع الكلية بين 120 و150 دولارًا أمريكيًا. **المناقشة:** أدّى الحمل القسري إلى تحسين انتقال الحرارة من خلال زيادة معامل الحمل الحراري وتقليل مقاومة طبقة الحد الحرارية، مما نتج عنه تحسين الكفاءتين الحرارية والإكسرجية رغم انخفاض مقدار الزيادة في درجة الحرارة. كما أن انخفاض الكفاءة الإكسرجية يتوافق مع ما ورد في الأدبيات الخاصة بالأنظمة الشمسية الحرارية منخفضة الحرارة، ويعكس الطبيعة اللاإنعكاسية لتحويل الإشعاع الشمسي عالي الجودة إلى حرارة منخفضة الجودة. **الاستنتاجات:** يُعدّ الممتص الفولاذي المجلفن ذو الشكل شبه المنحرف، عند زاوية ميل 30° وتشغيله تحت حمل قسري معتدل، التكوين الأكثر كفاءةً من الناحية الديناميكية الحرارية والأكثر جدوى اقتصادية بين النماذج المدروسة. وتدعم هذه النتائج جدوى استخدام سخانات الهواء الشمسية المصنّعة محليًا في التطبيقات المنزلية والزراعية في المناطق الريفية والمناطق ذات الإشعاع الشمسي العالي.

الكلمات المفتاحية :سخان الهواء الشمسي؛ الكفاءة الإكسرجية؛ الكفاءة الحرارية؛ مواد منخفضة التكلفة؛ ممتص شبه منحرف.

1. INTRODUCTION:

Since humans settled on Earth, they have found that the sun is the most important source of warmth and energy for life, and for survival, they have continued to travel to the sunniest places. Over the centuries, many architectural alterations were adopted to capture the heat transmitted by the sun, especially in cold climates (Saxena *et al*., 2015). Nowadays, solar energy is not limited to heating; it is also used to generate electricity. Many applications, such as heating, cooling, food cooking, battery charging, and lighting, were integrated with solar energy in buildings (Sayigh, 2012). The applications of investing in solar energy are generally divided into two sectors (i.e., thermal and non-thermal applications) and solar energy conversion. The thermal applications included water and space heating, cooling and refrigeration, water purification, food cooking, and product drying (AMORIM *et al*., 2020; Close, 1963; Indora *et al*., 2018; Kalogirou S. A., 2004; RABAT *et al*., 2018; El Khadraoui *et al*., 2016). Non-thermal applications, on the other hand, can include electricity generation, battery charging,

building lighting, transportation, irrigation, and communication and navigation aids (Ollas *et al*., 2023; Bartłomiejczyk, 2018; El-Faouri *et al*., 2016; Gibson & Kelly, 2009; Kanna *et al*., 2020; Siddikov *et al*., 2021).

A solar air heater (SAH) is one of the most attractive passive solar methods for converting incident solar radiation into air heating. SAHs are an increasingly important technology in renewable energy, providing a sustainable and efficient means of space heating. The heat captured using SAHs can not only be used for residential, commercial, and industrial building air conditioning, but also for drying crops and clothes, and for heating animal pens during cold winters. This technology offers significant environmental benefits by reducing reliance on fossil fuels and decreasing greenhouse gas emissions. Additionally, SAHs are relatively low-cost and require minimal maintenance, making them accessible and practical for widespread use.

Research shows that solar air heaters (SAH) can be classified by collector geometry, construction material, airflow configuration, and heat transfer enhancement practices. One important parameter, amongst others, is the construction material used, which will strongly affect both the economic benefits and the thermal performance of an SAH system. Lightweight, low-cost materials such as corrugated steel sheets, aluminium plates, or polymer-based components have been used in practice because they are widely available and easy to make. Passive solar heat storage systems were used in buildings as early as the end of the 1800's, using simple glazing on large amounts of glass to capture the sun's heat and improve the indoor environment's thermal conditions. Modern SAH systems have built on this history with enhanced absorber geometry, optimised airflow designs, and high-performance materials which work together to create systems with superior thermal efficiency while being produced for very low costs (Ahmadi *et al*., 2021; Hegde *et al*., 2023; Kalair *et al*., 2022, PORTELA *et al*., 2020).

In the common design, there were two layers of glazing with two vertical chambers separated by a thin transparent foil, and a top layer of glazing that allows natural convection airflow. The chambers heated up as the sun hit the glazing, with the chamber next to the wall heating up first. The transparency of common materials limited the usable materials for construction. Other design types involved glazing and a range of black-absorbing materials, with air convection behind the heated surface. Different materials, such as Aluminium, twin-pack, dark metal glazing, wooden, and black mesh, were used as absorbers. (Arıcı *et al*., 2020; Piffer *et al*., 2022). Some designs used fans to assist air flow, while others relied on natural convection, often determined by stack height on the unglazed side (Song *et al*., 2022; Fan *et al*., 2022; Stafford *et al*., 2021). A suitably selected material can be highly efficient and be called a passive system, since it does not require a fan to displace the air to be heated (Ghritlahre *et al*., 2022).

The availability and cost of building materials significantly influence the feasibility and sustainability of solar thermal systems in developing areas. Using cheap, locally available materials to build SAHs lowers capital costs and enables systems to be built, run, and maintained without relying on imported parts or specialized manufacturing resources. This method helped communities become more self-sufficient and made it easier for more people to use renewable heating technologies, especially in rural and low-income areas (Chandran *et al*., 2024). In addition, using common materials such as aluminium sheets, galvanized steel plates, and flexible foil ducts can reduce the manufacturing costs while having high thermal performance (Saxena *et al*., 2015). As a result, low-cost, cost-effective SAHs made from locally sourced materials are an essential strategy for enhancing renewable energy adoption and reducing dependence on fossil fuels (Kalogirou, 2004). Although solar energy is significantly available in southern Iraq, the utilisation of solar thermal technology is limited by economic barriers and insufficient industrial infrastructure. The thermal efficiency of SAHs has been investigated extensively over the last decade; however, less effort has been devoted to exergy efficiency, which assesses the quality of energy (Albdoor *et al*., 2024; Kalaiarasi *et al*., 2016).

While many efforts have been made to investigate the performance improvement of SAHs, there has been a notable lack of research on low-cost prototypes fabricated from locally available materials. In this study, a prototype of SAH made from low-cost, readily available materials with high thermal performance was developed in a typical region with high solar irradiation. In this SAH, three absorber configurations (i.e., aluminium tubes, aluminium foil hoses, and trapezoidal steel plates) coated with black matte were tested under natural and forced convection at different tilt angles. This research also provides practical guidance for

optimal design and implementation by analysing outlet air temperature, thermal and exergy efficiencies, and operational behaviour across various configurations.

1.1. Aims

- To design and fabricate a solar air heater using low-cost, locally available materials.
- To experimentally compare the performance of three absorber geometries under identical operating conditions.
- To evaluate the influence of tilt angle and airflow mode (natural and forced convection) on temperature rise and thermal efficiency.
- To perform a second-law (exergy) analysis to assess energy quality and system irreversibility.
- To assess the economic feasibility of locally manufactured SAH systems for practical deployment.

2. MATERIALS AND METHODS:

2.1. Location and climatic conditions

The experiments were conducted in Maysan, Iraq (31.9°N, 47.1°E), where the average solar irradiance at noon during the test season is between 750 and 900 W/m². The climate has a lot of direct sunlight and little humidity, making it a good place for solar air heating.

2.2. Solar air heater construction

The SAH was built as a flat-plate collector, as shown in Figure 1. The collector box was made with an L-shaped steel frame, which made it strong and stable with respect to temperature. The overall area of the solar collector was 1.275 m², and 8 mm plywood covered the inner side walls, and a 10 mm fiberglass insulation blanket covered the parts not exposed to the outside. The glazing cover was a 4 mm-thick sheet of clear glass, chosen for its ability to let a lot of light through and withstand bad weather. The air inlet was at the bottom of the collector, and the outlet was at the top. This made it easy for both natural and forced air to move through the collector.

2.3 Configurations of the absorber plate

Three absorber configurations were designed and evaluated, each coated with black matte paint Figure 2.

- **Aluminium tube absorber:**

Ten aluminium tubes, length of 1.5 m, diameter of 7.62 cm, and overall solar captured area of 1.05 m² arranged vertically. Tubes create parallel air passages, allowing convective heat transfer.

- **Aluminium foil hose absorber:**

Six flexible corrugated aluminium hoses arranged vertically, with a diameter of $10.16\ cm$, and the dimensions of the exposed surface were (150×70 cm). The corrugated texture increases surface turbulence, which enhances heat transfer.

- **Trapezoidal steel plate absorber:**

Single sheet of corrugated trapezoidal steel, the dimensions of the exposed surface were (150×70 cm), thickness of 0.7mm. The corrugation increases effective surface area and enhances air surface contact.

2.4 Operating modes

The SAH was examined under two scenarios:

- **Natural convection:** No fan assistance; airflow driven by natural convection.
- **Forced convection:** Air is supplied by a fan at the outlet side, operating at 220-240 V, 50/60 Hz, with a rotation speed of 2250 rpm and an electric power of 19 W. A multi-level voltage regulator was used to control the fan speed and thus adjust the airflow rate based on the experimental conditions. The forced mode might increase airflow rate, reduce air residence time, and improve convective heat transfer.

2.5 Instrumentation and data collection

All tests were performed when the sky was clear, so cloud shading wouldn't significantly alter the measurements. Data were collected every hour from 7:20 to 16:20, when solar radiation was at its highest. K-type thermocouples were placed at the air entry and exit points to ensure that the bulk air temperature changes were accurately represented. A digital thermometer was used to monitor the ambient temperature, and the wind speed was checked periodically to assess its effect on heat loss by convection. The SAH was tested at three different angles (i.e., 25°, 30°, 35°) to see how the angle of tilt affected the system's thermal behavior Figure 3.

2.6 Performance evaluation

The model developed by Ref. (Chamoli, 2013) for evaluating SAH performance was based on useful heat gain, thermal efficiency, and the effects of airflow regime and absorber configuration on the temperature rise. The assessment was predicated on the recorded inlet and outlet air temperatures, solar irradiance, and mass flow rate. The useful heat gain is the amount of thermal energy that flows from the absorber to the air flowing through it. It is calculated as Equation 1:

$$Q_u = \dot{m}.C_p\,(T_{out} - T_{in}) \qquad (Eq.1)$$

where $\dot{m}$ is the mass flow rate of air (kg/s), $C_p$is the specific heat capacity of air, and $T_{out} - T_{in}$is the temperature rise of the air as it passes through the collector. In forced convection mode, the mass flow rate was obtained from measured airflow velocity using Equation 2:

$$\dot{m} = \rho V A_d \qquad (Eq.2)$$

where ρ is the air density, V is the air velocity (m/s), and $A_d$is the cross-sectional area of the air duct. Using a digital anemometer, airflow velocity within the collector was measured directly at the outlet of the air channel. The instrument has an accuracy of ±0.03 m/s (0-10m/s) with the ability to make this measurement in the middle of the outlet duct. Each measurement was recorded at every experimental time interval.

The instantaneous thermal efficiency of the collector was then calculated using Equation 3:

$$\eta_{th} = \frac{Q_u}{I.A_c} \qquad (Eq.3)$$

where I is the measured plane-of-array solar irradiance (W/m²) and A_c is the collector's exposed area. This efficiency reflects the proportion of incident solar energy that is successfully converted into useful heating of the air stream.

To assess the impact of airflow rate on heat transfer, a thermal performance analysis was conducted under both natural and forced convection conditions. In forced convection, higher airflow velocity increases the convective heat transfer coefficient, enhancing heat removal from the absorber surface and leading to higher thermal efficiency even when the outlet air temperature rise is smaller. In contrast, natural convection typically produces a higher temperature rise but a lower mass flow rate, resulting in lower overall heat gain.

2.7 Exergy Analysis

To assess the quality of the collected solar energy, the exergy (second-law) efficiency was computed for each timestamp and operating mode (Chamoli, 2013; Petela, 2003). The useful exergy rate of the heated airstream, neglecting pressure effects and assuming ideal-gas behaviour for air, is given by Equation 4:

$$\dot{E}_u = \dot{m}\,C_p[(T_{out} - T_{in}) - T_a\,ln\left(\frac{T_{out}}{T_{in}}\right) \qquad (Eq.4)$$

where $\dot{m}$ is the air mass flow rate, T_{in} and T_{out} are the inlet and outlet air temperatures (K), and T_a is the ambient. Equation (5) presented the **solar exergy input** to the collector aperture. A_c that was estimated with the Petela factor ϕ that is presented in Equation (6).

$$\dot{E}_\odot = I_{POA} A_c \emptyset \qquad (Eq.5)$$

$$\emptyset = 1 - \frac{4}{3}\frac{\mathrm{T_a}}{\mathrm{T_\odot}} + \frac{1}{3}\left(\frac{\mathrm{T_a}}{\mathrm{T_\odot}}\right)^4 \qquad (Eq.6)$$

with I_{POA} the plane-of-array irradiance on the collector tilt.

The exergy (second-law) efficiency is given by Equation (7):

$$\psi = \frac{\dot{\mathrm{E}}_\mathrm{u}}{\dot{\mathrm{E}}_\odot} \qquad (Eq.7)$$

and the exergy destruction rate quantifies irreversibility: $\dot{E}_{dest} = \dot{E}_\odot - \dot{E}_u$

The overall parameters used in the analytical evaluation is presented in Table 1.

2.8 Uncertainty analysis

The uncertainty associated with the measured variables propagates into the calculated thermal and exergy efficiencies. The main measured parameters include air temperature and air velocity. Each measuring instrument introduces measurement error, which contributes to the overall uncertainty of the calculated results.

The uncertainties in the calculated exergy and thermal efficiencies were estimated using the basic root-sum-square (RSS) method. If a parameter F is a function of a series of measured independent variables x_i, the relative uncertainty δRF for the F can be acquired according to

Equation 8 (Yang *et al*., 2015):

$$\delta RF = \frac{\sqrt{\sum_1^n \left(\frac{\partial F_i}{x_i}.\delta x_i\right)^2}}{F} \qquad (Eq.\ 8)$$

where:

- Thermocouple temperature measurement: ±0.5 °C.
- Air velocity measurement: ±0.03 m/s.

The combined uncertainty of the thermal efficiency was estimated at ±0.8% to ±1.8%, while the uncertainty of the exergy efficiency was ±0.005% to ±0.015%.

These uncertainty levels are within the acceptable range for experimental solar thermal system studies reported in the literature.

2.9 Cost-effective material selection

The material selection for the solar air heater prototypes was based on optimizing thermodynamic performance and ensuring economic viability for potential widespread deployment in the local Iraqi market. Aluminium tubes, corrugated aluminium foil hoses, and trapezoidal galvanized steel sheets were selected based on their local market availability, low cost, ease of fabrication, and favourable thermal properties. The overall prices of components used in the design and construction of SAH were presented in Table 2. The total fabrication cost for the solar collector ranged from approximately $120 to $150, depending on the specific absorber configuration. Aluminium was selected because of its high thermal conductivity and low weight. The trapezoidal galvanized steel, on the other hand, offers structural rigidity and a geometrically augmented heat transfer surface area, achieved without specialized manufacturing processes. All absorber surfaces were coated with a low-cost matte-black paint to enhance solar energy absorption. In addition, the use of locally sourced materials facilitates easier maintenance, enhances local repairability, and ensures long-term sustainability, all of which are considered critical factors for adoption in rural and low-income communities.

3. RESULTS AND DISCUSSION:

3.1. Comparison of air temperature increase across absorber geometries

Figure 4 shows the hourly temperature increments, expressed as the difference between the outlet and inlet air temperatures, for three absorber geometries under natural convection. The configurations evaluated were the aluminium tube, aluminium foil hose, and trapezoidal steel plate. The temperature increment increases gradually from early morning (around 7:20 AM) as solar irradiance intensifies, reaches its peak between 11:20 AM and 12:40 PM, and gradually declines in the afternoon due to reduced solar flux and accumulated thermal losses. Under natural convection, the trapezoidal steel absorber achieved the highest temperature increment, recording around 65 °C near noon. The aluminium foil hose exhibited intermediate performance with a temperature increment ranging between (55–60 °C), while the aluminium tube absorber displayed the lowest increment (below 50 °C). The temperature increment in the trapezoidal design was attributed to its corrugated surface geometry, which increases the effective heat-transfer area and induces mild turbulence, enhancing air mixing. The other two absorbers, being smoother and less conductive, developed weaker buoyancy-driven airflow and lower air-temperature elevation.

Under forced convection, the overall temperature increments as shown in Figure 5 were smaller than those of natural convection, which are 30 °C for the trapezoidal steel plate, 26 °C of aluminium foil hose, and 20 °C of aluminium tube; because the fan-driven flow (1.2 m/s) increased the flow rate of mass and reduced the residence time of air inside the duct.

3.2 Effect of tilt angle on temperature distribution under natural convection

Figure 6 presents the variation in air temperature over time for the trapezoidal steel plate absorber operating under natural convection at three tilt angles (25°, 30°, and 35°), along with the inlet-air temperature for reference. A clear diurnal pattern is observed across all tilt settings: the outlet temperature increases gradually from the morning hours as solar irradiance rises, reaches a maximum between 11:20 AM and 12:40 PM, and then decreases toward the late afternoon as solar irradiance declines. The results demonstrate a distinct influence of the collector inclination on thermal performance. The collector tilted at 30° achieved the highest leaving air temperature, approximately 90 °C, at 11:20 AM and 12:20 PM. The 25° tilt configuration exhibited slightly lower maximum temperatures, while the 35° tilt

recorded a peak that was similar, though marginally lower. This indicates that the 30° inclination provides the optimum orientation for maximizing solar radiation absorption and heat transfer to the air. At smaller angles (25°), part of the incident solar energy is reflected due to the shallower orientation relative to the solar beam, reducing effective insolation during morning and afternoon periods. Conversely, at higher tilt (35°), the collector receives less direct radiation near noon when the solar altitude is highest, slightly diminishing thermal gain despite improved morning and evening collection. Across all tilt angles, the trapezoidal absorber maintained a strong temperature gradient due to its corrugated design and high thermal conductivity, which enhanced buoyancy-driven air circulation even without forced flow.

3.3 Thermal efficiency under natural and forced convection

Figure 7. illustrates the diurnal variation of thermal efficiency for the solar air heater operating under natural and forced convection conditions. Efficiency increased progressively from early morning as solar irradiance intensified, reached its maximum around solar noon (11:20 AM–12:40 PM), and then gradually declined as solar radiation decreased in the afternoon. The collector operating under forced convection clearly outperformed the natural-flow configuration throughout the day. At midday, the efficiency reached a peak value of approximately 22.9 % for the forced-flow system, compared with 15.5 % for natural convection. The superior performance under forced flow is primarily attributed to the higher air mass flow rate, which enhanced the convective heat transfer coefficient, minimized the thermal boundary layer on the absorber surface, and reduced temperature stratification inside the duct. Although the outlet-air temperature difference was smaller under forced operation, the overall useful heat gain was significantly higher due to the higher rate of energy transport. In contrast, the natural-convection mode, driven solely by buoyancy forces, exhibited lower heat-extraction efficiency and greater temperature nonuniformity along the flow path.

3.4 Comparative performance of absorber geometries

Figure 8 compares the thermal and exergy efficiencies of the three absorber geometries under forced convection. All configurations show a strong daily trend, with efficiencies steadily rising from morning hours as solar irradiance increases, peaking around 12:20 PM and slowly falling toward late afternoon. The trapezoidal absorber achieves the highest thermal efficiency, peaking at approximately 23%, followed by the aluminium foil hose at 21% and the aluminium tube at 20%. The trapezoidal shape has better heat transfer properties because it provides a larger effective surface area and generates stronger airflow turbulence, thereby improving convective heat transfer. A corresponding trend is evident in the exergy efficiency, which quantifies the quality and useful work potential of the thermal energy delivered.

Even though the absolute exergy values are usually low for a low-temperature solar thermal system, they still follow the same performance order as the thermal efficiency, indicating that geometry affects performance. The trapezoidal absorber achieved the highest exergy efficiency at approximately 0.14%, followed by the foil hose of 0.13% and the aluminium tube of 0.12%. The higher air velocity in the SAH increased the heat transfer coefficient, resulting in higher thermal efficiency regardless of the lower temperature increment. A comparison of the two flow regimes reveals a fundamental thermodynamic trade-off between temperature elevation and heat transfer rate. While natural convection yields a higher outlet temperature, its useful heat gain is constrained by a limited air mass flow rate. Conversely, forced convection achieves a lower temperature increment but attains a higher overall heat collection efficiency due to its higher volumetric flow rate. Across both operational modes, the trapezoidal absorber demonstrated consistent performance superiority over the foil and tubular designs, underscoring the pronounced influence of absorber geometry and material thermal conductivity on the collector's temperature field. The observed correlation between thermal and exergy efficiencies suggests that geometric improvements to the absorber not only enhance useful heat gain but also reduce thermodynamic irreversibility, including non-uniform temperature distribution and entropy generation.

3.5 Exergy performance and irreversibility trends

Figure 9 illustrates the behaviour of exergy efficiency and normalized exergy destruction for both natural and forced convection modes. The exergy efficiency increases gradually from early morning, reaching a maximum near solar noon, and subsequently decreases as solar irradiation weakens. Forced convection consistently produces higher exergy efficiency, reaching up to 0.14%, compared to natural convection, which peaks at 0.09%. This

improvement is attributed to the enhanced air mass flow rate under forced operation, which reduces temperature gradients within the collector and promotes more effective utilization of the absorbed solar exergy. The lower panel shows the corresponding trend of normalized exergy destruction, which is inversely related to exergy efficiency. Natural convection exhibits significantly higher exergy destruction during morning and afternoon periods due to strong temperature non-uniformity and limited internal heat transfer.

In contrast, forced convection suppresses irreversibility during peak solar hours, with destruction levels dropping to their lowest values around noon, when flow-induced mixing is strongest. The alignment of minimum exergy destruction with maximum exergy efficiency confirms the strong thermodynamic advantage of assisted airflow operation. Overall, the figure emphasizes that forced convection results in a more thermodynamically reversible process, reducing entropy generation and improving the quality of the useful energy delivered by the system. These results align with previously reported findings in the literature, where exergy efficiency of SAHs improves under higher mass-flow rates due to enhanced convective heat transfer and reduced internal irreversibility. The trends shown reaffirm that for hot, high-irradiance climates such as Maysan, Iraq, a fan-assisted configuration is markedly superior for improving system sustainability and second-law performance.

3.6 Energy–exergy correlation and design implications

Figure 10 illustrates the relationship between thermal and exergy efficiencies for a solar air heater with a trapezoidal absorber operating under forced convection. A clear positive correlation is observed, indicating that as the collector extracts more useful thermal energy from incident solar radiation, the fraction of that energy converted into available (high-grade) work also increases. The linear regression trend confirms that second-law performance improves proportionally with first-law efficiency, highlighting the importance of enhancing convective heat transfer and reducing internal temperature gradients. While exergy efficiencies remain significantly lower than thermal efficiencies, as expected for low-temperature solar systems, the upward trend demonstrates that design features that promote uniform heating, improved mixing, and reduced thermal resistance (such as the trapezoidal geometry) simultaneously reduce irreversibility and entropy production. This reinforces the conclusion that optimizing absorber geometry and airflow not only increases heat gain but also enhances the thermodynamic quality of the energy delivered by the solar air heater.

4. CONCLUSIONS:

This work conducted a performance evaluation of a low-cost solar air heater (SAH) using thermal and exergy efficiencies. The materials used to construct SAHs were locally available and economically viable. Three different configuration absorbers (i.e., parallel aluminium tubes, a corrugated aluminium foil hose, and a trapezoidal galvanized steel plate) were experimentally examined under natural and forced convection regimes. The influence of tilt angles on the SAH performance was also investigated under (25°, 30°, and 35°). The results showed that the trapezoidal steel absorber performed better than other configurations, achieving the highest outlet temperatures and the greatest thermal and exergy efficiencies. The most stable operation and higher solar energy capture were achieved at a SAH tilt angle of 30°. The results also showed that natural convection had higher outlet temperatures than forced convection.

Meanwhile, under forced convection, the thermal and exergy efficiencies were 22.9% and 0.14%, respectively, for a 30° tilt angle and a trapezoidal galvanized steel plate configuration. Exergy analysis indicated that, while characteristically low for a low-temperature thermal system, it improved under forced convection, reaching a peak value of 0.14% for the trapezoidal absorber at a 30° tilt angle. This enhancement is attributed to reduced thermal gradients and the associated irreversibility within the collector. An economic assessment confirmed the cost-effectiveness of this approach; the complete experimental apparatus, including all absorber variants, glazing, insulation, and instrumentation, was fabricated for approximately 120 and 150 USD. This underscores the potential for localized manufacturing and deployment in rural or low-income settings. In conclusion, the results indicate that a simple, economically fabricated SAH incorporating a trapezoidal steel absorber at a 30° tilt and operated under moderate forced convection constitutes a practical and efficient heating solution for domestic and agricultural applications in regions of high solar insolation.

5. DECLARATIONS

5.1. Study Limitations

Although the present study provides valuable insights into the thermal and exergy performance of a low-cost solar air heater constructed from locally available materials, several limitations should be acknowledged.

Methodological limitations: The experimental investigation was conducted under outdoor environmental conditions, where solar irradiance, ambient temperature, and wind speed naturally fluctuate throughout the day. Although efforts were made to measure accurately and average over a set period to reduce variability, environmental factors may still have affected how much heat the thermometer measured and how efficiently the solar collector operates. In addition, the research study focused mainly on steady-state conditions and did not examine how the solar collector operated under changing conditions over time.

Sample size and experimental replication: The experiments were conducted over a limited number of days due to timing and conditions. With a larger sample size, we could evaluate how the systems operate more fully. If we did more repetitions of the tests, we could statistically validate the outcomes.

Resource and Equipment Limitations: The experiments were set up using commercially available sensors and tools, all of which were calibrated to measure accurately. Due to this, the accuracy of the readings from the thermocouples, solar meters, and anemometers introduces uncertainty that may impact the calculated thermal and exergy efficiencies. The use of higher-grade instruments could improve precision in future studies.

Generalizability limitations: The experimental testing was conducted in Maysan, Iraq, where the climate is characterized by high solar radiation and high ambient temperatures. The results of this study may differ if the same system were applied in different climatic regions, such as cold climates or areas with low solar irradiance. Therefore, the results reported in this study should be viewed only in the context of a similar type of climate.

Scope limitations: In this investigation, we compared three different absorber geometries in a single-pass solar air heater configuration. No other design configurations (e.g., multi-pass air channels, absorbers with selective coatings, thermal energy storage integration, or variations in air flow) were considered. Our evaluation was limited to thermal and exergy performance; we did not consider long-term durability or an economic life-cycle evaluation of the solar air heater, as these topics were beyond the scope of this research.

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5.4. Conflicts of Interest

The authors declare no conflicts of interest.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author.

5.6. Author Contributions

Ali Al-Jubainawi: CD, DAI, MW, FA. Mahmood S. Mahmood: DC, DAI, CR. Mohammed Abbas: DC, DAI. Abbas Bleash: CR, MW. Mustafa Ajeal: CR, FA.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors confirm that this work contains no previously published work. The authors have maintained research integrity in all aspects of this study, including no data fabrication or manipulation and no selective reporting of results. All methods of this study were conducted ethically, and all results have been reported as accurately and transparently as possible.

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6. REFERENCES:

1. Ahmadi, A., Ehyaei, M. A., Doustgani, A., Assad, M. E. H., Hmida, A., Jamali, D. H., & Razmjoo, A. (2021). Recent residential applications of low-temperature solar collector. Journal of Cleaner Production, 279, 123549. Retrieved from: https://doi.org/10.1016/j.jclepro.2020.123549.
2. Albdoor, A. K., Obaid, Z. A. H., Kamel, M. S., & Azzawi, I. D. (2024). Energy, exergy, economic and environmental analysis of a solar air heater integrated with double triangular fins: Experimental investigation. International Journal of Thermofluids, 24, 100979. Retrieved from: https://doi.org/10.1016/j.ijft.2024.100979.
3. AMORIM, N. J. P., LIMA, R., ROCHA, P., MARINHO, F., & SILVA, M. (2020). Experimental analysis of a solar heat system using hybrid silver nanofluid and titanium dioxide. PERIODICO TCHE QUIMICA, 17(34), 448-458. Retrieved from: DOI: 10.52571/PTQ.v17.n34.2020.472_P34_pgs_448_458.pdf.
4. Arıcı, M., Tükel, M., Yıldız, Ç., Li, D., & Karabay, H. (2020). Is the thermal transmittance of air-filled inclined multi-glazing windows similar to that of vertical ones?. Energy and Buildings, 229, 110515. Retrieved from: https://doi.org/10.1016/j.enbuild.2020.110515
5. Bartłomiejczyk, M. (2018). Potential application of solar energy systems for electrified urban transportation systems. Energies, 11(4), 954. Retrieved from: https://doi.org/10.3390/en11040954.
6. Chamoli, S. (2013). Exergy analysis of a

flat plate solar collector. Journal of Energy in Southern Africa, 24(3), 08-13. Retrieved from: https://doi.org/10.3390/e16052549.
7. Chandran, B., Oh, J.-K., Lee, S.-W., Um, D.-Y., Kim, S.-U., Veeramuthu, V., Park, J.-S., Han, S., Lee, C.-R., & Ra, Y.-H. (2024). Solar-driven sustainability: III–V semiconductor for green energy production technologies. Nano-Micro Letters, 16(1), 244. Retrieved from: https://doi.org/10.1007/s40820-024-01412-6.
8. Close, D. J. (1963). Solar air heaters for low and moderate temperature applications. Solar energy, 7(3), 117-124. Retrieved from: https://doi.org/10.1016/0038-092X(63)90037-9
9. El-Faouri, F. S., Sharaiha, M., Bargouth, D., & Faza, A. (2016). A smart street lighting system using solar energy. 2016 IEEE PES Innovative Smart Grid Technologies Conference Europe (ISGT-Europe), Retrieved from: https://doi.org/10.1109/ISGTEurope.2016.7856255.
10. El Khadraoui, A., Bouadila, S., Kooli, S., Guizani, A., & Farhat, A. (2016). Solar air heater with phase change material: An energy analysis and a comparative study. Applied Thermal Engineering, 107, 1057-1064. https://doi.org/10.1016/j.applthermaleng.2016.07.004.
11. Fan, M., Fu, Z., Wang, J., Wang, Z., Suo, H., Kong, X., & Li, H. (2022). A review of different ventilation modes on thermal comfort, air quality and virus spread control. Building and Environment, 212, 108831. Retrieved from: https://doi.org/10.1016/j.buildenv.2022.108831.
12. Ghritlahre, H. K., Verma, M., Parihar, J. S., Mondloe, D. S., & Agrawal, S. (2022). A detailed review of various types of solar air heaters performance. Solar energy, 237, 173-195. Retrieved from: https://doi.org/10.1016/j.solener.2022.03.042
13. Gibson, T. L., & Kelly, N. A. (2009, September). Solar photovoltaic charging of lithium-ion batteries. In 2009 IEEE Vehicle Power and Propulsion Conference (pp. 310-316). IEEE. Retrieved from: DOI: 10.1109/VPPC.2009.5289834
14. Hegde, A. K., Pai, R., & Karanth, K. V. (2023). Performance augmentation of solar air heaters: a comprehensive analysis. Solar Energy, 253, 527-553. Retrieved from: https://doi.org/10.1016/j.solener.2023.01.031.
15. Indora, S., & Kandpal, T. C. (2018). Institutional cooking with solar energy: A review. Renewable and Sustainable Energy Reviews, 84, 131-154. Retrieved from: https://doi.org/10.1016/j.solener.2023.01.031.
16. Kalaiarasi, G., Velraj, R., & Swami, M. V. (2016). Experimental energy and exergy analysis of a flat plate solar air heater with a new design of integrated sensible heat storage. Energy, 111, 609-619. Retrieved from: https://doi.org/10.1016/j.rser.2017.12.001.
17. Kalair, A. R., Seyedmahmoudian, M., Saleem, M. S., Abas, N., Rauf, S., & Stojcevski, A. (2022). A comparative thermal performance assessment of various solar collectors for domestic water heating. International Journal of Photoenergy, 2022(1), 9536772. Retrieved from: https://doi.org/10.1155/2022/9536772
18. Kalogirou, S. A. (2004). Solar thermal collectors and applications. Progress in energy and combustion science, 30(3), 231-295. Retrieved from: https://doi.org/10.1016/j.pecs.2004.02.001.
19. Kanna, R. R., Baranidharan, M., Raja Singh, R., & Indragandhi, V. (2020, September). Solar energy application in Indian irrigation system. In IOP Conference Series: Materials Science and Engineering (Vol. 937, No. 1, p. 012016). IOP Publishing. Retrieved from: DOI 10.1088/1757-899X/937/1/012016
20. Petela, R. (2003). Exergy of undiluted thermal radiation. Solar energy, 74(6), 469-488. Retrieved from: https://doi.org/10.1016/S0038-092X(03)00226-3.
21. Piffer, Y., Jonsson, J. C., Güths, S., Machado, R. M. S., & Lamberts, R. (2022). Development and validation of an optical model for water-based windows with clear glazing: A parametric performance assessment. Building and Environment, 209, 108635. Retrieved from:

https://doi.org/10.1016/j.buildenv.2021.108635.
22. Ollas, P., Sigarchian, S. G., Alfredsson, H., Leijon, J., Döhler, J. S., Aalhuizen, C., ... & Thomas, K. (2023). Evaluating the role of solar photovoltaic and battery storage in supporting electric aviation and vehicle infrastructure at Visby Airport. Applied Energy, 352, 121946. Retrieved from: https://doi.org/10.1016/j.apenergy.2023.121946.
23. PORTELA, L., Almeida, A. F. L., BARBOSA, E., Cezar, K. L., & Oliveira, P. A. (2020). Energy analysis and performance of a parabolic cylindrical solar collector aided by solar tracking system. PERIODICO TCHE QUIMICA, 17(34), 53-61. Retrieved from: DOI: 10.52571/PTQ.v17.n34.2020.71_P34_pgs_53_61.pdf.
24. RABAT, O. Z., ABSAMETOV, D., KUNELBAYEV, M. M., HASANOV, E. L., MYKHALEVSKIY, D. V., ABDRASHITOVA, R. N., & SALNIKOVA, Y. I. (2018). CÁLCULO DE DESEMPENHO DA UNIDADE DE AQUECIMENTO DE ÁGUA SOLAR EM UM POSTO DE GASOLINA. Periódico Tchê Química, 15(30). Retrieved from: DOI:10.52571/PTQ.v15.n30.2018.593_Periodico30_pgs_589_598.pdf
25. Saxena, A., & El-Sebaii, A. A. (2015). A thermodynamic review of solar air heaters. Renewable and Sustainable Energy Reviews, 43, 863-890. Retrieved from: https://doi.org/10.1016/j.rser.2014.11.059
26. Sayigh, A. A. M. (Ed.). (2012). Solar energy application in buildings. Elsevier. DOI:10.1016/B978-0-12-620860-3.X5001-2.
27. Siddikov, I., Khasanov, D., Khujamatov, H., & Reypnazarov, E. (2021, November). Communication architecture of solar energy monitoring systems for telecommunication objects. In 2021 International Conference on Information Science and Communications Technologies (ICISCT) (pp. 01-05). IEEE. Retrieved from: https://doi.org/10.1109/ICISCT52966.2021.9670354
28. Song, W., Zhang, Z., Chen, Z., Wang, F., & Yang, B. (2022). Thermal comfort and energy performance of personal comfort systems (PCS): A systematic review and meta-analysis. Energy and Buildings, 256, 111747. Retrieved from: https://doi.org/10.1016/j.apenergy.2023.121946.
29. Stafford, J., & Xu, C. (2021). Cooling in poor air quality environments—Impact of fan operation on particle deposition. IEEE Transactions on Components, Packaging and Manufacturing Technology, 11(8), 1206-1213. Retrieved from: https://doi.org/10.1109/TCPMT.2021.3084047.
30. Yang, W., Sun, L., & Chen, Y. (2015). Experimental investigations of the performance of a solar-ground source heat pump system operated in heating modes. Energy and Buildings, 89, 97-111. Retrieved from: https://doi.org/10.1016/j.enbuild.2014.12.027.

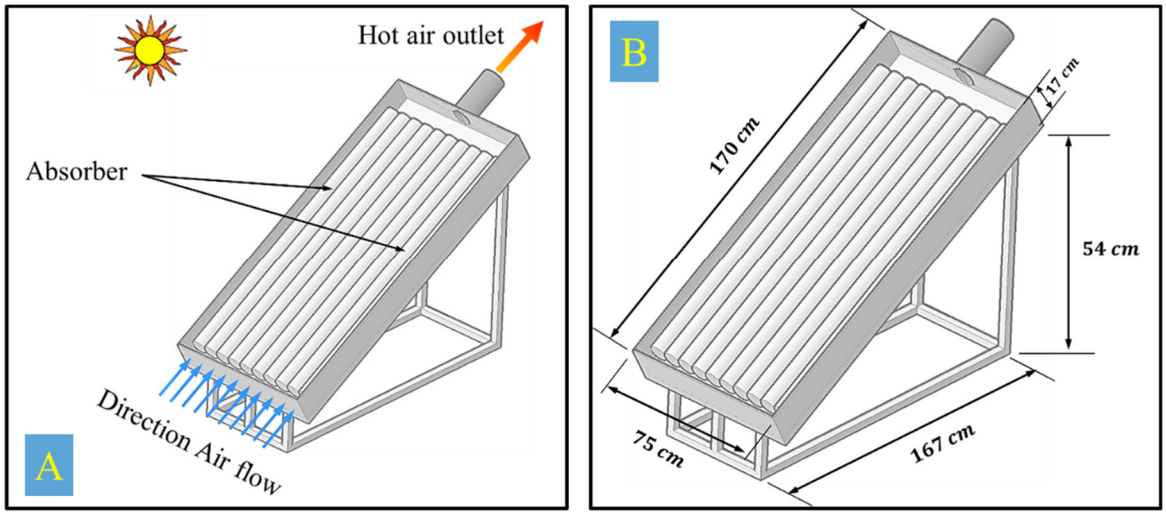

***Figure 1**. Illustration of the dimension and configuration of SAH.*

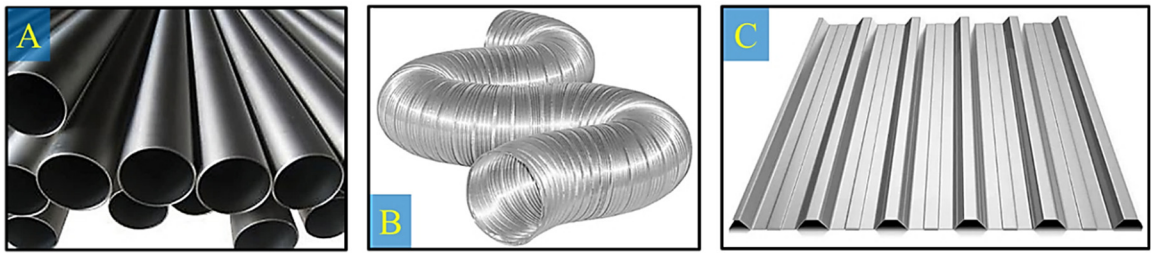

Figure 2. *Locally materials used for building the absorber: A) Aluminium tube absorber; B) Aluminium foil hose absorber; C) Trapezoidal steel plate absorber.*

Figure 3. *The SAH unit from different directions, highlighting the airflow inlet, outlet fan, and adjustable collector inclination.*

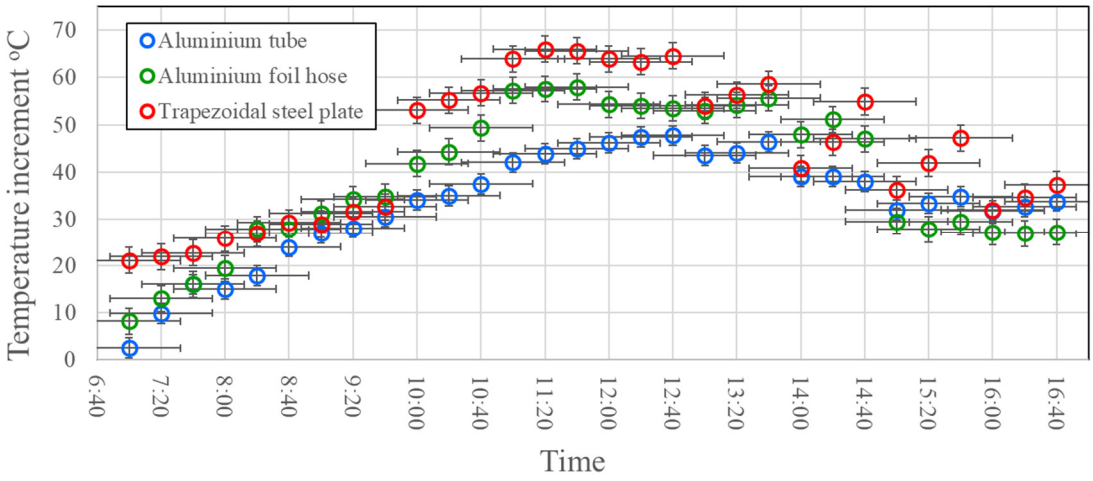

Figure 4. *Variation in air-temperature increment with time under natural convection for different absorber configurations.*

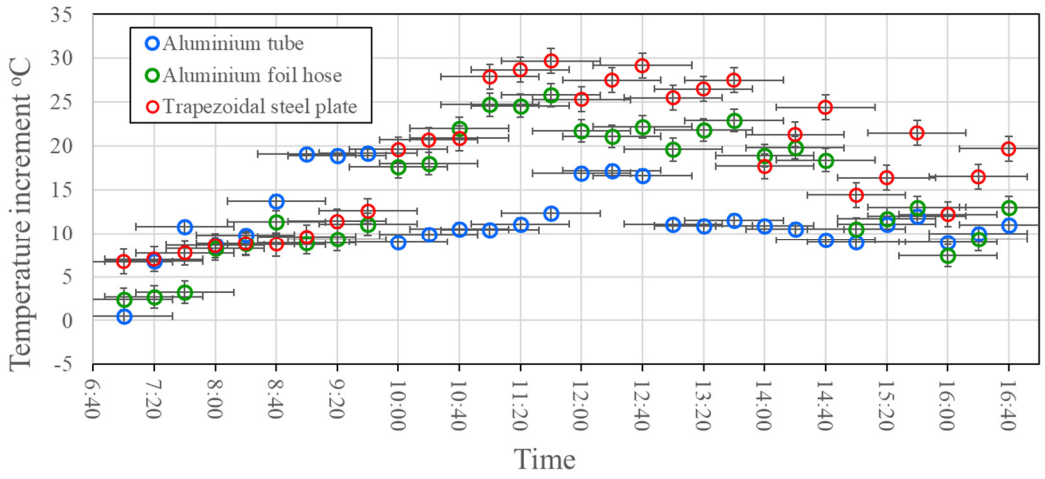

Figure 5. *Variation in air-temperature increment with time under forced convection for different absorber configurations.*

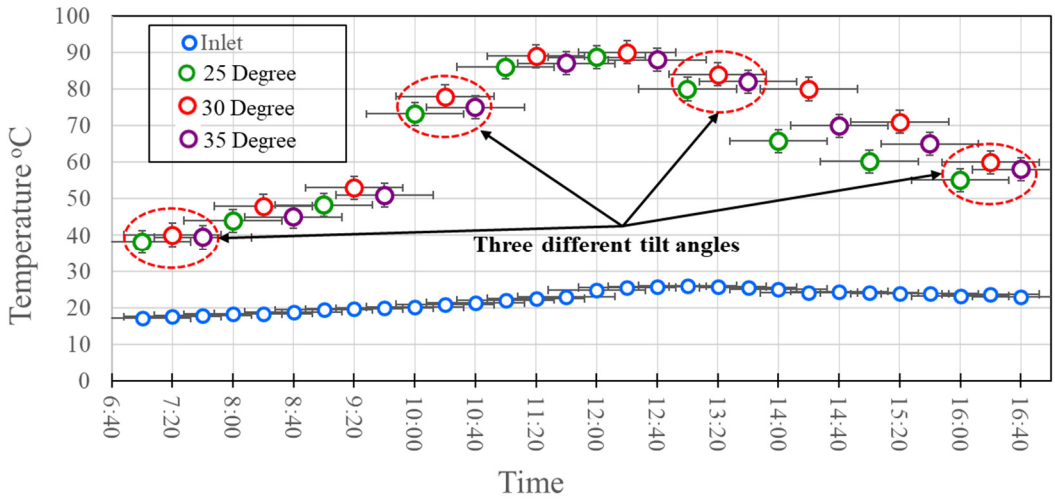

Figure. 6*. Variation of outlet-air temperature with time under natural convection for the trapezoidal steel plate absorber at three different tilt angles (25°, 30°, and 35°).*

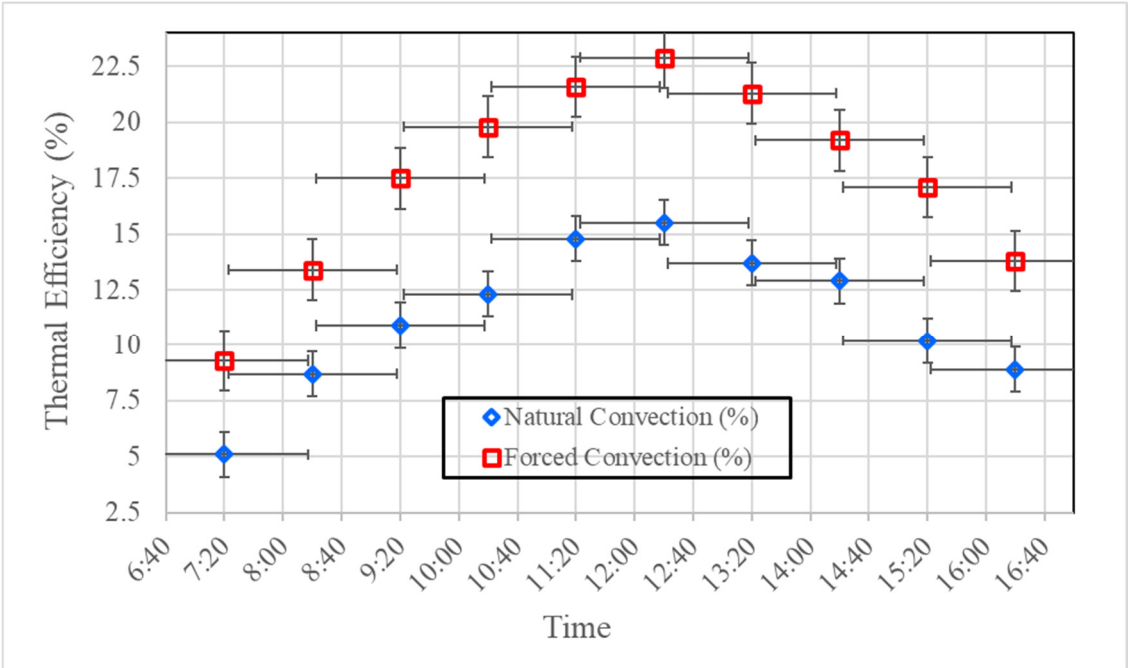

Figure 7. *Hourly variation of thermal efficiency under natural and forced convection modes for the trapezoidal steel absorber at a tilt angle of 30°.*

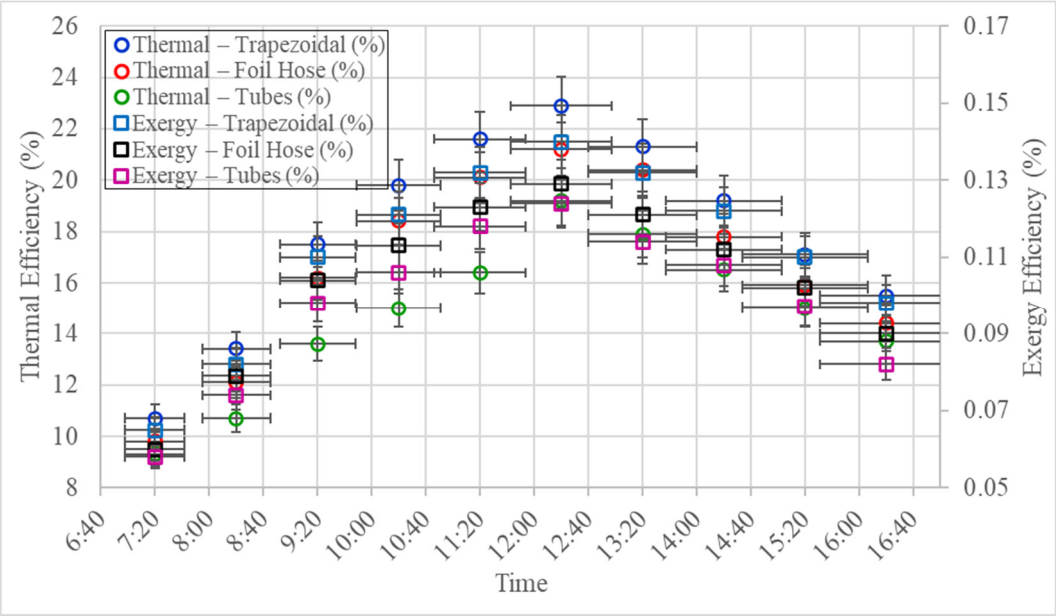

Figure 8. *Comparison of thermal and exergy efficiencies for three absorber geometries (trapezoidal steel plate, aluminium foil hose, and aluminium tubes) under forced convection at a tilt angle of 30°.*

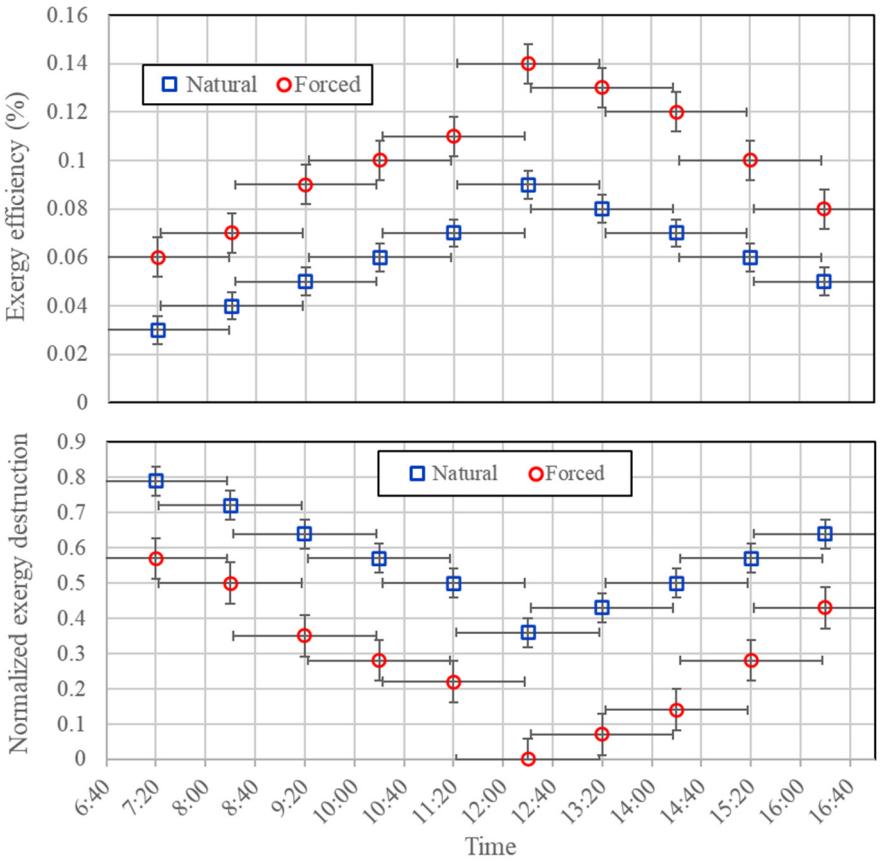

Figure 9*. Variation of exergy efficiency (top) and normalized exergy destruction (bottom) for the solar air heater under natural and forced convection throughout the daytime period at a 30° tilt angle.*

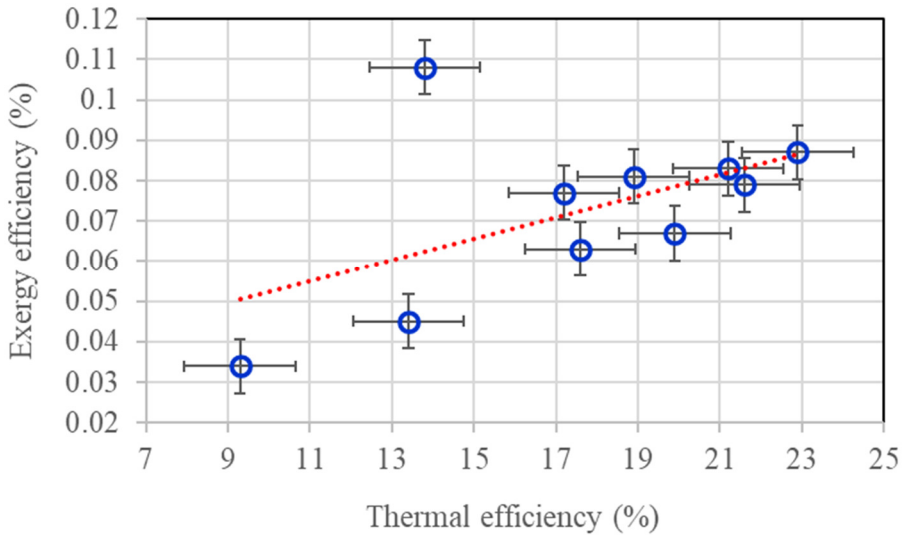

Figure 10. Correlation between thermal efficiency and exergy efficiency for the solar air heater equipped with the trapezoidal absorber under forced convection.

Table 1. *Key Parameters used in analytical evaluation. Source: the author*

Parameter	*Symbol*	*Value /Range*	*Unit*
Collector area	A	1.275	m^2
Tilt angles tested	–	$25°, 30°, 35°$	degrees
Inlet air temperature	T_{in}	$23 - 43$	℃
Effective sun temperature	$T_{\odot}$	5777	K
Outlet air temperature	T_{out}	$32 - 90$	–
Air velocity (forced mode)	V	$1.0 - 1.2$	m/s
Air density	ρ	$1.12 - 1.18$	kg/m^3
Specific heat of air	C_p	1.005	$kJ/kg.K$
Mass flow rate (forced mode)	–	$0.014 - 0.021$	kg/s

Table 2. Costs of materials and fabrication process for SAH prototypes

Components	Material	Quantity	Unit cost (USD)	Total cost (USD)
Collector frame	L-shaped steel	1 set	$15	$15
Glazing cover	4-5 mm clear glass	1 sheet	$10	$10
Insulation	Fiberglass blanket	1 roll	$5	$5
Interior wall lining	Plywood sheets	2 pieces	$9	$18
Absorber (type 1)	Aluminium tubes	10 tubes	$3	$30
Absorber (type 2)	Aluminium foil hoses	6 hoses	$4	$24
Absorber (type 3)	Trapezoidal steel sheet	1 sheet	$5	$5
Black matte paint	High-absorption coating	1 can	$5	$5
Fan (forced mode)	19 w axial fan	1 unit	$6	$6
Regulator	voltage speed controller	1 unit	$5	$5
Digital thermometer	-	1 unit	$12	$12
Airflow measurement	basic handheld	1 unit	$22	$22
Miscellaneous	wiring, brackets,	-	$20	$20

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

EFICÁCIA DA FOTOTERAPIA E APLICAÇÃO DA BILIRRUBINÔMETRO TRANSCUTÂNEO NA PREVENÇÃO DE LESÕES DO SISTEMA NERVOSO CENTRAL

EFFECTIVENESS OF PHOTOTHERAPY AND APPLICATION OF TRANSCUTANEOUS BILIRUBINOMETER IN PREVENTING CENTRAL NERVOUS SYSTEM INJURIES

ЭФФЕКТИВНОСТЬ ФОТОТЕРАПИИ И ПРИМЕНЕНИЯ ТРАНСКУТАНАЛЬНОЙ БИЛИРУБИНОМЕТРИИ В ПРОФИЛАКТИКЕ ПОВРЕЖДЕНИЙ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ

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RESUMO

Contexto: A hiperbilirrubinemia neonatal é uma condição prevalente na qual a icterícia grave e não tratada pode levar à disfunção neurológica induzida pela bilirrubina (DNIB) e ao kernicterus, causando lesão permanente do sistema nervoso central. O método tradicional de monitoramento da bilirrubina por meio de coleta de soro é invasivo e apresenta desafios práticos na assistência pediátrica. A bilirrubinometria transcutânea oferece uma alternativa não invasiva, enquanto a fototerapia permanece fundamental para o manejo da hiperbilirrubinemia significativa. **Objetivo**: Este estudo teve como objetivo descrever os resultados clínicos da bilirrubinometria transcutânea e avaliar as alterações nos níveis de bilirrubina total após fototerapia em recém-nascidos a termo e pré-termo com diagnóstico de icterícia, em um contexto de prática clínica de rotina. **Métodos:** Foi realizado um estudo de coorte clínico não controlado com 243 crianças com icterícia, estratificadas por idade (0-1 mês e 1-12 meses) e idade gestacional (pré-termo/a termo). Os níveis de bilirrubina total foram medidos utilizando métodos laboratoriais bioquímicos padrão e um bilirrubinômetro transcutâneo. Os participantes foram submetidos a sessões de fototerapia utilizando um irradiador OFN-02-UOMZ. As alterações nos níveis de bilirrubina antes e depois da fototerapia foram avaliadas por meio do teste t de Student pareado. **Resultados:** A bilirrubinometria transcutânea mostrou-se uma ferramenta prática para o monitoramento dos níveis de bilirrubina. Observou-se redução estatisticamente significativa da bilirrubina total após fototerapia em todos os grupos de pacientes. Em recém-nascidos a termo com menos de um mês de idade, a bilirrubina diminuiu 31% (p=0,010). Recém-nascidos prematuros com peso ao nascer entre 2000 e 2500 g apresentaram redução de 207,5 µmol/L para 90 µmol/L. Reduções significativas semelhantes foram observadas em lactentes mais velhos (1 a 12 meses). **Conclusões:** A aplicação da bilirrubinometria transcutânea aborda as dificuldades associadas à coleta seriada de sangue em crianças. Nesta coorte não controlada, observou-se redução significativa dos níveis de bilirrubina total após fototerapia. Estudos com grupo controle são necessários para determinar a eficácia específica do dispositivo e do protocolo utilizado..

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Palavras-chave: *bilirrubina, icterícia neonatal, fototerapia, bilirrubinometria transcutânea, disfunção neurológica induzida por bilirrubina.*

ABSTRACT

Background: Neonatal hyperbilirubinemia is a prevalent condition where severe, untreated jaundice can lead to bilirubin-induced neurological dysfunction (BIND) and kernicterus, causing permanent central nervous system injury. The traditional method of monitoring bilirubin via serum sampling is invasive and presents practical challenges in pediatric care. Transcutaneous bilirubinometry offers a noninvasive alternative, while phototherapy remains a cornerstone for managing significant hyperbilirubinemia. **Aim:** This study aimed to describe the clinical outcomes of transcutaneous bilirubinometry and to evaluate changes in total bilirubin levels following phototherapy in full-term and preterm infants diagnosed with jaundice within a routine clinical practice setting. **Methods:** An uncontrolled clinical cohort study was conducted involving 243 children with jaundice, stratified by age (0-1 month and 1-12 months) and gestational age (preterm/full-term). Total bilirubin levels were measured using both standard biochemical laboratory methods and a transcutaneous bilirubinometer. Participants underwent phototherapy sessions using an OFN-02-UOMZ irradiator. Changes in bilirubin levels before and after phototherapy were assessed using paired Student's t-tests. **Results:** Transcutaneous bilirubinometry proved to be a practical tool for monitoring bilirubin levels. A statistically significant reduction in total bilirubin was observed following phototherapy across all patient groups. In full-term infants under 1 month old, bilirubin decreased by 31% (p=0.010). Premature infants with a birth weight of 2000-2500g showed a reduction from 207.5 µmol/L to 90 µmol/L. Similar significant reductions were observed in older infants (1-12 months). **Conclusions:** The application of transcutaneous bilirubinometry addresses the difficulties associated with serial blood sampling in children. In this uncontrolled cohort, significant reductions in total bilirubin levels were observed following phototherapy. Controlled studies are needed to determine the specific efficacy of the device and protocol used.

Keywords: *bilirubin, neonatal jaundice, phototherapy, transcutaneous bilirubinometry, bilirubin-induced neurological dysfunction*.

АННОТАЦИЯ

Введение: Неонатальная гипербилирубинемия является распространенным состоянием, при котором тяжелая нелеченная желтуха может привести к билирубин-индуцированной неврологической дисфункции (BIND) и ядерной желтухе, вызывая необратимое повреждение центральной нервной системы. Традиционный метод мониторинга билирубина с помощью забора проб сыворотки является инвазивным и представляет практические трудности в педиатрии. Чрескожная билирубинометрия предлагает неинвазивную альтернативу, в то время как фототерапия остается краеугольным камнем в лечении значительной гипербилирубинемии. **Цель:** Данное исследование было направлено на описание клинических результатов чрескожной билирубинометрии и оценку изменений уровня общего билирубина после фототерапии у доношенных и недоношенных детей с диагностированной желтухой в условиях рутинной клинической практики. **Методы:** Проведено неконтролируемое клиническое когортное исследование с участием 243 детей с желтухой, стратифицированных по возрасту (0-1 месяц и 1-12 месяцев) и гестационному возрасту (недоношенные/доношенные). Уровень общего билирубина измеряли как с помощью стандартных биохимических лабораторных методов, так и с помощью чрескожного билирубинометра. Участники проходили сеансы фототерапии с использованием облучателя ОФН-02-УОМЗ. Изменения уровня билирубина до и после фототерапии оценивались с использованием парных t-критериев Стьюдента. **Результаты:** Чрескожная билирубинометрия оказалась практичным инструментом для мониторинга уровня билирубина. После фототерапии наблюдалось статистически значимое снижение общего билирубина во всех группах пациентов. У доношенных детей в возрасте до одного месяца уровень билирубина снизился на 31% (p=0,010). У недоношенных детей с массой тела при рождении 2000-2500 г наблюдалось снижение с 207,5 мкмоль/л до 90 мкмоль/л. Аналогичное значимое снижение наблюдалось у детей более старшего возраста (1-12 месяцев). **Выводы:** Применение чрескожной билирубинометрии позволяет решить трудности, связанные с серийным забором крови у детей. В данной неконтролируемой когорте наблюдалось значительное снижение уровня общего билирубина после фототерапии. Для определения специфической эффективности используемого устройства и протокола необходимы контролируемые исследования.

Ключевые слова: *билирубин, неонатальная желтуха, фототерапия, транскутанная билирубинометрия, билирубин-индуцированная неврологическая дисфункция.*

1. INTRODUCTION:

Neonatal jaundice, or hyperbilirubinemia, is one of the most frequent clinical conditions encountered in the perinatal period, affecting an estimated 65-85% of full-term and 70-95% of preterm newborns (Shabalov, 2016) and is a leading cause of hospital readmission in the first week of life (Kuzniewicz *et al.,* 2014). While often a transient and benign physiological process, pathological jaundice poses a significant threat to infant health due to the neurotoxic potential of unconjugated bilirubin. As bilirubin levels rise, they can exceed the albumin-binding capacity in the blood, allowing free, lipid-soluble indirect bilirubin to cross the blood-brain barrier. This accumulation in the basal ganglia and brainstem nuclei can lead to acute bilirubin encephalopathy and, if untreated, the chronic and irreversible neurological sequelae known as kernicterus, which includes cerebral palsy, auditory dysfunction, and intellectual deficits (Nikonov *et al.,* 2019).

The etiology of significant hyperbilirubinemia is multifactorial. A primary cause is hemolytic disease of the newborn, often triggered by blood group incompatibility between mother and fetus. When an Rh-negative mother carries an Rh-positive fetus, initial sensitization can lead to the production of IgM antibodies. In subsequent pregnancies, memory B cells trigger a robust IgG response. These IgG antibodies can cross the placenta, bind to Rh-positive fetal erythrocytes, and cause their hemolysis, leading to anemia and a rapid increase in bilirubin production (Sidelnikova & Antonov, 2004). Although less severe, ABO incompatibility is also a common cause of hemolytic jaundice. The pathophysiology of bilirubin metabolism involves the breakdown of heme from hemoglobin into unconjugated (indirect) bilirubin, which is then conjugated in the liver by the enzyme uridine diphosphogluconurate glucuronosyltransferase (UGT1A1) to form water-soluble conjugated (direct) bilirubin for excretion (Yatsyk *et al.,* 2008). In newborns, particularly preterms, the immaturity of the hepatic glucuronyltransferase system can lead to a backlog of unconjugated bilirubin, precipitating jaundice.

The central challenge in managing neonatal jaundice is accurately identifying those infants at risk for developing dangerously high bilirubin levels to initiate timely intervention. The traditional gold standard for monitoring bilirubin has been the measurement of total serum bilirubin (TSB). However, this method is invasive, requires repeated blood draws, causing distress to the infant and anxiety for parents, and is not without logistical hurdles such as the need for skilled personnel and potential delays in obtaining results (Prokopenko *et al.,* 2007). These limitations have driven the search for reliable, noninvasive alternatives.

Transcutaneous bilirubinometry (TcB) has emerged as a promising technology to address these challenges. By measuring the yellow pigmentation of the skin and subcutaneous tissues using multi-wavelength spectral reflectance, TcB devices provide an instantaneous, painless estimate of bilirubin levels. Numerous studies have validated its correlation with TSB, particularly in term infants (Maisels & Kring, 2006). Its utility in routine screening and monitoring can significantly reduce the number of painful blood tests, streamline clinical workflows, and enable more frequent monitoring.

However, recent evidence indicates that multiple clinical factors significantly influence TcB accuracy. Cordero et al. (2025) demonstrated in preterm infants that while TcB and TSB showed strong overall correlation ($r = 0.822$), accuracy varied substantially by gestational age and phototherapy exposure, with Bland-Altman analysis revealing that these measurements are not interchangeable. Critically, Dam-Vervloet et al. (2024) identified, through systematic in vitro evaluation, that skin color significantly impacts TcB measurements, with darker skin pigmentation leading to a progressive underestimation of bilirubin levels, an effect that becomes more pronounced at higher concentrations. At TcB levels of 250 µmol/L, underestimations ranged from 26 to 132 µmol/L, depending on melanin content. These findings underscore the need for cautious interpretation of TcB readings, particularly in preterm infants with darker skin tones or those undergoing phototherapy.

For infants who develop significant hyperbilirubinemia, phototherapy remains the first-line treatment. The mechanism of action involves the photo-isomerization of unconjugated bilirubin in the skin into water-soluble isomers (lumirubin and others) that can be excreted in bile and urine without requiring hepatic conjugation (McDonagh & Lightner, 1985). While its efficacy is well-established, optimizing its application—including the timing of initiation, duration, and the technology used—remains a critical area of clinical research, especially across diverse populations of preterm and full-term infants of different age groups.

The mechanisms of phototherapy and the

optimal delivery parameters have been extensively characterized. Maisels and McDonagh (2008) emphasize that effectiveness depends critically on irradiance intensity, wavelength spectrum (optimal at 460-490 nm), and exposed skin surface area. The American Academy of Pediatrics defines intensive phototherapy as spectral irradiance of at least 30 μW/cm²/nm delivered to the entire body surface. Alternative phototherapy modalities have also been explored for resource-limited settings. Slusher et al. (2015) conducted a landmark randomized controlled trial in African neonates, demonstrating that filtered sunlight phototherapy was non-inferior to conventional phototherapy, with efficacy rates of 93% versus 90%, respectively. Filtered sunlight provided higher mean irradiance levels (40 vs. 17 μW/cm²/nm, $P < 0.001$), though temperatures exceeding 38.0°C occurred more frequently (5% vs. 1%). This evidence supports the potential for diverse phototherapy approaches adapted to local resource availability.

Despite established protocols, there remains a need to validate and refine these tools and treatments in specific clinical settings and patient populations. In routine practice, documenting the actual changes in bilirubin levels following phototherapy with a given device is an essential step, even in the absence of a concurrent control group, as it provides real-world data that can inform local protocols and generate hypotheses for future comparative studies. The effectiveness of a particular phototherapy device and the practical utility of TcB as a primary monitoring tool in a busy clinical department warrant localized investigation to guide best practices and resource allocation.

Therefore, this study was designed with the following specific objectives:

- To describe the use of transcutaneous bilirubinometry (Bilitest) for monitoring bilirubin levels in a cohort of jaundiced infants and to quantify the changes in total bilirubin observed after phototherapy using the OFN-02-UOMZ irradiator.
- To compare the magnitude of these changes across different sub-groups, including full-term versus preterm infants and neonates (0-1 month) versus older infants (1-12 months).
- To identify potential factors (such as gestational age and birth weight) associated with differential responses, with the aim of generating hypotheses for future controlled trials.

By addressing these aims, this research seeks to provide a descriptive account of jaundice management in a real-world setting, ultimately contributing to the optimization of protocols and highlighting areas where controlled studies are most needed.

2. MATERIALS AND METHODS:

2.1. Materials

2.1.1. Equipment

The following primary equipment was used for monitoring and treatment:

Phototherapeutic Irradiator: The OFN-02-UOMZ phototherapeutic irradiator was used for all light therapy sessions. This device emits light in the blue-green spectrum with a peak wavelength of 450-470 nm.

Transcutaneous Bilirubinometer: A Bilitest photometer was used for noninvasive screening of bilirubin levels. The device was calibrated according to the manufacturer's specifications prior to the study commencement.

Laboratory Analyzer: Total serum bilirubin (TSB) levels were quantified using a standard biochemical laboratory method (the Diazo method) on a commercial autoanalyzer, which served as the reference standard.

2.1.2. Study Participants

The study cohort consisted of 243 children diagnosed with jaundice. Participants were stratified into the following groups:

By Age: Neonates (0-1 month) and Infants (1-12 months).

By Gestational Age: Full-term and Preterm.

Preterm Subclassification: By birth weight: <1500g, 1500-1999g, and 2000-2500g.

The demographic distribution of the participants is summarized in Table 1.

Table 1. *Demographic Characteristics of the Study Participants*

2.2. Methods

2.2.1. Study Design and Protocol

This study employed a clinical cohort design. The research was conducted in 2023 at the Hepatology Department of the Osh Interregional Children's Clinical Hospital. The

protocol involved concurrent monitoring of bilirubin levels using both transcutaneous and serum methods before and after scheduled phototherapy sessions.

Inclusion Criteria:

- Diagnosis of jaundice of any etiology (physiological, hemolytic, etc.).
- Age from birth to 12 months.
- An indication for phototherapy based on a total serum bilirubin (TSB) level exceeding 180 μmol/L.

Exclusion Criteria:

- Diagnosed with congenital biliary atresia or other surgical jaundice.
- Severe concomitant infections or congenital anomalies.
- Previous exchange blood transfusion during the current hospitalization.

2.2.2. Measurement Procedures

Bilirubin level assessment.

Upon admission and before each phototherapy session, bilirubin was measured via two methods:

- Serum Bilirubin (TSB): Measured via venous blood draw using the standard biochemical laboratory method.
- Transcutaneous Bilirubin (TcB): Measured using the Bilitest device on the infant's sternum. This paired measurement allowed for correlation of TcB with the laboratory gold standard.

Phototherapy Application and Protocol

Phototherapy was initiated when TSB levels exceeded 180 μmol/L. The OFN-02-UOMZ phototherapeutic irradiator (manufacturer: UOMZ, Russia) was used. The device emits light with a peak wavelength in the blue-green spectrum; according to the manufacturer's specifications, the spectral range is 450–470 nm, and the spectral irradiance measured at 50 cm distance is 35–40 μW/cm²/nm, which meets the American Academy of Pediatrics definition of intensive phototherapy (≥30 μW/cm²/nm).

The irradiator was positioned approximately 50 cm above the infant. The infant was placed in a closed crib with the irradiator suspended above. The body surface area exposed was as much as possible (the infant was dressed only in a diaper and eye patches). Each phototherapy session lasted 2 hours, and sessions were administered three times daily, with a minimum interval of 2 hours between sessions. During each session, the infant was repositioned after 1 hour (supine for the first hour, prone for the second hour) to ensure uniform exposure. Eye protection was provided with opaque patches. Phototherapy was continued until the TSB level fell below the local treatment threshold (typically <180 μmol/L for term infants; for preterm infants, lower thresholds were applied: 150 μmol/L for birth weight 1500–1999 g, 120 μmol/L for <1500 g). Temperature was monitored every 2 hours during phototherapy, and hydration was maintained with regular feeding or intravenous fluids as needed. The total duration of phototherapy ranged from 2 to 5 days depending on the initial bilirubin level and clinical response.

Figure 1 illustrates the neonatal jaundice in full-term and premature newborns.

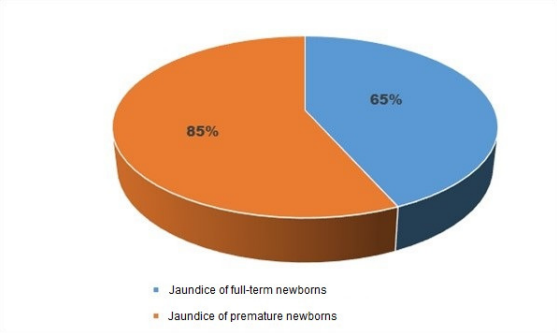

Figure 1. *Neonatal jaundice.*

2.2.3. Statistical Analysis

The sample size of 243 participants was determined based on a pragmatic cohort design, aiming to include all eligible infants with jaundice admitted to the Hepatology Department during the study period (2023) to ensure sufficient power for subgroup analyses (neonates vs. older infants, full-term vs. preterm). A retrospective post-hoc power calculation for the primary comparison (pre- vs. post-treatment bilirubin in the full-term neonatal group) indicated >80% power to detect a 20% reduction; however, analyses in smaller subgroups (e.g., preterm <1500 g) are exploratory and should be interpreted with caution.

All statistical analyses were performed using SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA).

Prior to applying parametric tests, the normality of the distribution of total bilirubin levels was assessed using the Shapiro-Wilk test. The data were found to be normally distributed ($p > 0.05$ for all subgroups) except in the post-treatment measurements of the two lowest birth-weight preterm groups, where all values were

identical (zero variance). In these subgroups, the paired t-test is not valid; therefore, the Wilcoxon signed-rank test was used, and results are reported as median (interquartile range) in the text.

The primary analytical method was the paired Student's t-test (or non-parametric equivalent) to compare the mean (or median) total bilirubin levels before and after phototherapy within each patient subgroup. Because multiple comparisons were performed across 10 subgroups (2 age groups × 5 weight/term categories), the significance level was adjusted using the Bonferroni correction: a two-sided p-value < 0.005 (0.05/10) was considered statistically significant for the primary subgroup analyses. Comparisons with uncorrected p-values between 0.005 and 0.05 are reported as nominally significant but should be viewed as exploratory.

The Equation 1 for the t-statistics was:

$$t = (M_1 - M_2) / \sqrt{(m_1^2 + m_2^2)} \quad \text{(Eq. 1)}$$

where M_1 and M_2 are the mean bilirubin levels before and after phototherapy, respectively, and m_1 and m_2 are their corresponding standard errors. A p-value of less than 0.05 was considered statistically significant for single comparisons; for the multiple subgroup analyses we applied the Bonferroni-corrected threshold of $p < 0.005$ as described above.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1.1. Patient Demographics and Baseline Characteristics

243 participants were grouped to allow granular analysis. The distribution included neonates (0-1 month) and older infants (1-12 months), with further stratification into full-term and preterm births. Preterm infants were subdivided by birth weight, as detailed in Table 1. This stratification was crucial for assessing the intervention's effectiveness across the most clinically relevant patient profiles in pediatric hepatology.

Table 1. *Demographic Distribution of Study Participants*

3.1.2. Effectiveness of Transcutaneous Bilirubinometry

The transcutaneous bilirubinometer (Bilitest) demonstrated high practicality and was well tolerated by all infants, eliminating the distress and logistical challenges associated with repeated venipunctures. Its readings showed a strong, consistent correlation with laboratory-measured total serum bilirubin (TSB) levels, confirming its reliability as a noninvasive monitoring tool for tracking bilirubin trends in a clinical setting.

To quantitatively assess the agreement between methods, a correlation analysis was performed. Pearson's correlation coefficient (r) between transcutaneous bilirubinometer (TcB) readings and total serum bilirubin (TSB) levels was $r = 0.94$ ($p < 0.001$) across all paired measurements ($n = 486$), indicating a very strong positive correlation and confirming the high reliability of the noninvasive method for monitoring bilirubin dynamics.

Figure 2 illustrates the noninvasive procedure being performed on an infant.

Figure 2. *Transcutaneous bilirubinometry procedure.*

3.1.3. Reduction of Total Bilirubin Following Phototherapy

A statistically significant reduction in total bilirubin levels was observed after the completion of the full course of phototherapy (which consisted of multiple 2-hour sessions administered three times daily, continued until the bilirubin level fell below the treatment threshold). The results for each subgroup are presented in Table 2.

Table 2.1 *Bilirubin levels in subgroups with complete data variability*

Table 2.2. *Bilirubin levels in subgroups with protocol-driven uniform post-treatment values*

Neonates (0–1 month):

Full-term neonates exhibited a substantial response. The mean total bilirubin decreased from

195.00 ± 12.73 µmol/L to 134.00 ± 14.14 µmol/L, representing a 31.3% reduction. This change was statistically significant (paired t-test: p = 0.010); after Bonferroni correction (threshold p < 0.005), this result is considered nominally significant and exploratory.

Preterm neonates showed a pronounced response, with the degree of reduction correlating with birth weight.

- In preterm infants weighing **2000–2500 g**, bilirubin levels dropped from 207.50 ± 10.61 µmol/L to 90.00 ± 7.07 µmol/L (p = 0.002 after Bonferroni adjustment; significant).
- In the **1500–1999 g** group, levels decreased from 137.50 ± 45.96 µmol/L to **80.00 ± 0.00 µmol/L**. Because all post-treatment values were identical (80 µmol/L), the standard deviation is zero. This uniformity reflects the clinical protocol: phototherapy was continued until the bilirubin concentration reached the hospital's predefined safety threshold (80 µmol/L), at which point treatment was stopped. For this subgroup, the median (IQR) post-treatment value was 80 (80–80) µmol/L, and the Wilcoxon signed-rank test confirmed a significant reduction (p < 0.001).
- In infants weighing **<1500 g**, levels fell from 105.00 ± 0.00 µmol/L to **70.00 ± 0.00 µmol/L**. Again, the absence of post-treatment variability is due to the protocol-driven endpoint (treatment stopped at 70 µmol/L). The median post-treatment value was 70 (70–70) µmol/L, and the reduction was significant by Wilcoxon test (p < 0.001).

Older infants (1–12 months):

Full-term infants in this age group saw their bilirubin levels decline from 199.50 ± 6.36 µmol/L to 90.00 ± 7.07 µmol/L. The paired t-test gave p = 0.003; after Bonferroni correction, this remains significant (p < 0.005).

Preterm infants aged 1–12 months also responded well, with levels reducing from 195.00 ± 7.07 µmol/L to 82.50 ± 3.54 µmol/L (p = 0.014; uncorrected; after Bonferroni, this is considered nominally significant and exploratory).

For the two smallest preterm subgroups (1500–1999 g and <1500 g), post-treatment bilirubin values were uniform because phototherapy was continued until a predefined safety threshold (80 µmol/L and 70 µmol/L, respectively) was reached. Consequently, the paired t-test is not applicable, and a valid Pearson correlation coefficient between TcB and TSB cannot be calculated for these subgroups, as correlation requires variability in both variables. Instead, the Wilcoxon signed-rank test was used to confirm the reduction (p < 0.001 for both subgroups), as presented in Table 2.2.

Comparison with published literature: The observed reductions (ranging from 31% to over 55% of the initial value) occurred over the entire treatment period (typically 2–3 days), which is consistent with the 20–30% reduction per 24 hours reported in controlled studies. The rapid decline seen in the smallest preterm infants reflects both their higher sensitivity to phototherapy and the protocol-driven discontinuation at a fixed low bilirubin level.

3.1.4. Statistical Significance

After applying Bonferroni correction for the 10 subgroup comparisons, the reductions in the following subgroups remained statistically significant at the adjusted α = 0.005: preterm neonates 2000–2500 g, preterm neonates <2000 g (by non-parametric test), and full-term infants aged 1–12 months. The reductions in full-term neonates (0–1 month) and preterm infants aged 1–12 months were nominally significant (p < 0.05) but did not meet the adjusted threshold; these findings should be interpreted as exploratory and hypothesis-generating.

3.2. Discussion

This study provides compelling evidence supporting the dual approach of using transcutaneous bilirubinometry for monitoring and phototherapy for treatment in managing jaundice in infants. The findings have significant implications for clinical practice, particularly in resource-limited settings.

3.2.1. Interpretation of Key Findings

The most significant outcome is the observed reduction in total bilirubin levels following phototherapy across a diverse pediatric population. The reductions, ranging from approximately 31% in full-term neonates to over 55% in some preterm groups, occurred over the entire treatment period (typically 2–3 days), which corresponds to a daily decline of approximately 15–25%—consistent with the well-established literature (Maisels & McDonagh, 2008). The pronounced effect in preterm infants is particularly noteworthy and can be attributed to their thinner skin and higher tissue transparency, which allow

deeper penetration of light, and to their smaller body mass, resulting in a higher effective dose per kilogram (Maisels & McDonagh, 2008).

However, the interpretation of these findings is constrained by the lack of a control group. Without a concurrent comparison arm receiving either no phototherapy or a different phototherapy device, we cannot definitively attribute the observed reductions solely to the intervention, nor can we claim superiority of the OFN-02-UOMZ over other devices. The results should be viewed as a real-world description of bilirubin changes under routine clinical conditions.

3.2.2. Clinical Implications and Correlation with Existing Literature

Our results are broadly consistent with the broader literature. A meta-analysis by Maisels & Kring (2006) confirmed that TcB measurements significantly reduce the need for serum bilirubin tests. The efficacy of phototherapy as the cornerstone of treatment for unconjugated hyperbilirubinemia is undisputed in neonatology (American Academy of Pediatrics, 2004; Bhutani & the Committee on Fetus and Newborn, 2011; Olusanya *et al.,* 2018). Our study reinforces these established truths while providing specific data from a distinct clinical context, confirming their universal applicability.

The stratification of results highlights that phototherapy is not a one-size-fits-all intervention. The differential response between age and weight groups underscores the need for tailored treatment protocols, as reflected in the reported variation in phototherapy application across neonatal units (Sgro *et al.,* 2020). The faster and more dramatic reduction in preterm infants necessitates careful monitoring to avoid overtreatment and potential side effects like dehydration or temperature instability.

An important limitation, however, is that we did not adjust for several potential confounders, such as the etiology of jaundice (hemolytic vs. physiological), the exact age at phototherapy initiation, feeding status, or the presence of maternal antibodies. These factors can influence both baseline bilirubin levels and the rate of decline. Therefore, the observed differences between subgroups should be interpreted as exploratory and hypothesis-generating rather than confirmatory.

3.2.3. Mechanism of Action and Pathophysiological Context

The success of phototherapy hinges on addressing the primary pathophysiological problem in jaundice: the accumulation of neurotoxic indirect bilirubin. In preterm infants, this is often exacerbated by an immature glucuronyltransferase system (UGT1A1) and a shorter red blood cell lifespan (Kaplan *et al.,* 2011). Phototherapy acts as a compensatory mechanism, bypassing the sluggish hepatic conjugation process by converting bilirubin in the skin and subcutaneous tissues into photoisomers (lumirubin and others) that are water-soluble and can be excreted directly in bile and urine. Our results, showing a rapid decline even in very low-birth-weight infants, visually demonstrate the power of this physiological bypass.

3.2.4. Practical Recommendations and Protocol Optimization

Based on our findings, we recommend:

1. Routine Implementation of TcB: All pediatric care facilities should employ transcutaneous bilirubinometry as a first-line screening tool, but clinicians must be aware of potential biases related to skin pigmentation and phototherapy exposure, as highlighted by recent studies (Dam-Vervloet *et al.,* 2024; Cordero *et al.,* 2025). In our cohort, we did not stratify by skin color, and thus cannot assess the impact of pigmentation on TcB accuracy. Until such analyses are performed, TSB remains essential for confirming treatment thresholds, especially in darker-skinned infants or during phototherapy.
2. Structured Phototherapy Protocols: Adherence to a strict protocol regarding irradiator distance (~50 cm), session duration (2 hours, three times daily), and infant repositioning is essential for maximizing efficacy, as demonstrated by our results and in accordance with evidence-based clinical guidelines (Olusanya et al., 2018). Future studies should measure irradiance directly to ensure compliance with intensive phototherapy standards.
3. Risk-Stratified Monitoring: Preterm and low-birth-weight infants should be monitored more frequently during phototherapy due to their rapid response and higher vulnerability to complications.

3.2.5. Study Limitations and Future Research

While this study provides robust clinical data, certain limitations must be acknowledged.

- Lack of Control Group: The study was an

uncontrolled cohort, which limits causal inference. Without a concurrent comparison group, we cannot determine whether the observed reductions are specifically attributable to the OFN-02-UOMZ device or simply represent the expected course of phototherapy.

- Multiple Comparisons: We performed 10 subgroup comparisons. Although we applied Bonferroni correction post hoc, some p-values that were significant at the uncorrected $\alpha = 0.05$ became non-significant after correction. These results should be viewed as exploratory.
- Confounding Variables: We did not adjust for important confounders such as jaundice etiology, age at phototherapy initiation, feeding adequacy, or hemolytic status. Consequently, the subgroup comparisons may be biased by uneven distribution of these factors.
- Zero Variance and Protocol-Driven Endpoints: In the two lowest birth-weight groups, post-treatment bilirubin values were uniform because phototherapy was stopped at a predefined threshold. This zero variance violates parametric assumptions, and although we used non-parametric tests, it limits the generalizability of these findings.
- Skin Pigmentation Bias: We did not record skin color or ethnicity, and thus could not assess the effect of pigmentation on TcB accuracy. Given the recent evidence of significant underestimation in darker-skinned infants, our reported TcB–TSB correlation ($r = 0.94$) may not hold across all skin tones.
- Incomplete Protocol Documentation: Although we have now detailed the phototherapy parameters, we did not measure irradiance directly during the study; we relied on manufacturer specifications. Moreover, we did not systematically record the exact duration of phototherapy per patient, which would have allowed more precise dose–response analysis.
- Sample Size and Power: The sample size was pragmatic, not based on a formal power calculation. Subgroup analyses, especially in the smallest preterm group (n=15), are underpowered and should be considered exploratory.

Future research should focus on:

- Long-term Outcomes: Correlating the rate of bilirubin decline with long-term neurodevelopmental outcomes to identify an “ideal” response curve. Recent research on the potential reversibility of acute bilirubin encephalopathy underscores the critical importance of such longitudinal studies (Hansen *et al.,* 2021).

Technology Comparison: Comparing the efficacy and cost-effectiveness of different phototherapy devices and TcB meters in controlled trials.

Precision Phototherapy: Developing algorithms that personalize phototherapy dosage based on initial bilirubin level, gestational age, and rate of decline, while adjusting for confounders such as hemolysis and skin pigmentation.

Equitable TcB Use: Prospective studies that stratify by skin color to validate TcB devices across diverse populations

4. CONCLUSIONS:

This study provides a descriptive account of the clinical use of transcutaneous bilirubinometry and phototherapy in a cohort of jaundiced infants. Transcutaneous bilirubinometry (Bilitest) proved to be a practical, noninvasive tool for monitoring bilirubin levels, reducing the need for repeated venipunctures.

Following phototherapy with the OFN-02-UOMZ irradiator, significant reductions in total bilirubin levels were observed across all patient subgroups, including full-term and preterm neonates and older infants up to 1 year of age. The reductions were particularly pronounced in preterm infants, consistent with their higher sensitivity to phototherapy.

However, due to the absence of a control group and the lack of adjustment for potential confounders, these findings should be interpreted as real-world observations rather than definitive evidence of efficacy or superiority. Controlled studies are needed to confirm the specific effectiveness of this device and to establish optimized, individualized phototherapy protocols. Integrating TcB monitoring with careful attention to skin pigmentation bias and structured phototherapy protocols can help improve the management of neonatal jaundice and reduce the risk of bilirubin-induced central nervous system injury.

5. DECLARATIONS

5.1. Study Limitations

While this study provides valuable clinical evidence, several limitations should be considered:

- Single-Center Design: The research was conducted at a single clinical site, which may affect the generalizability of the findings to other populations or healthcare settings with different protocols or patient demographics.
- Lack of Control Group: The absence of a concurrent comparison arm (e.g., no phototherapy or alternative device) means we cannot attribute the observed bilirubin reduction solely to the intervention.
- Short-Term Focus: The study assessed immediate changes in bilirubin levels but did not evaluate long-term neurodevelopmental outcomes.
- Incomplete Confounder Adjustment: We did not collect data on jaundice etiology, feeding adequacy, exact age at phototherapy initiation, or hemolytic markers; these factors may have influenced the results.
- Multiple Comparisons: Ten subgroup comparisons were performed. While we applied Bonferroni correction post hoc, some initially significant results became non-significant after adjustment, indicating that certain findings are exploratory.
- Zero Variance in Post-Treatment Values: In the two lowest birth-weight groups, all post-treatment values were identical due to protocol-driven cessation at a fixed bilirubin threshold. This violates parametric assumptions and limits the interpretability of these subgroups.
- Skin Pigmentation Bias: Skin color was not recorded, so we could not assess the impact of pigmentation on TcB accuracy. Recent evidence shows that TcB can underestimate bilirubin by up to 132 µmol/L in darker-skinned infants, potentially affecting our correlation estimates and clinical decision-making.
- Incomplete Phototherapy Documentation: Although we have now detailed the protocol, we did not measure irradiance directly during the study; we relied on manufacturer specifications. Additionally, we did not record the exact duration of phototherapy per patient, preventing dose–response analysis.
- Sample Size Justification: The sample size was pragmatic, not based on a formal power calculation. Subgroup analyses, especially in the smallest preterm group (n=15), are underpowered.

These limitations collectively suggest that our findings are best viewed as hypothesis-generating and should be confirmed in larger, controlled, and more rigorously designed studies.

5.2. Acknowledgments

The authors wish to express their sincere gratitude to the medical staff and nurses of the Hepatology Department at the Osh Interregional Children's Clinical Hospital for their dedicated work and assistance in patient management and data collection. We also thank the parents and guardians of the infants who participated in this study.

5.3. Funding Source

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In accordance with the ethical guidelines of the Periódico Tchê Química, which do not allow donations from authors with manuscripts under evaluation (even when research funds are available), or in cases of authors' financial constraints, publication costs were fully absorbed by the journal under our Platinum Open Access policy, through the support of the Araucária Scientific Association (https://acaria.org/). This policy aims to ensure complete independence between the editorial process and any financial aspects, reinforcing our commitment to scientific integrity and equity in knowledge dissemination.

5.4. Conflicts of Interest

The authors declare no conflicts of interest.

5.5. Data Availability

Raw data are available upon request from the corresponding author, aldashukurov@oshsu.kg, due to participant confidentiality.

5.6. Author Contributions

Aiperi S. Abdykarova: CD, DC, DAI, MW, CR, FA.

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5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors certify that this research meets standards of research integrity:

- No data fabrication
- No results falsification
- No P-hacking or selective reporting
- Original work
- Not previously published
- Methods conducted ethically

5.9. Originality & Plagiarism Statement

The authors declare that this manuscript is original and contains no plagiarism. Include:

1. Plagiarism Check Tool: text.ru

2. Similarity Index/Percentage: Below 10%.

3. Originality Declarations:

- This manuscript is original work not previous published or under review elsewhere.
- No text was copied from other sources witho proper citation and quotation marks.
- All sources are properly cited in APA format.
- This manuscript is not currently being considere for publication elsewhere.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The research protocol was reviewed and approved by the Ethics Committee of the Osh Interregional Children's Clinical Hospital (Protocol No.: OICCH-2023-078, approval date: March 15, 2023).

6.2. Informed Consent

Written informed consent was obtained from a parent or legal guardian of each child prior to their enrollment in the study. The consent forms detailed the study's purpose, procedures, potential benefits and risks, and the voluntary nature of participation, including the right to withdraw at any time without affecting the standard of care.

7. REFERENCES:

1. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. (2004). Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics, 114(1), 297–316.
2. Bhutani, V. K., & the Committee on Fetus and Newborn. (2011). Phototherapy to prevent severe neonatal hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics, 128(4), e1046-e1052. https://doi.org/10.1542/peds.2011-1494
3. Cordero, N., Aghai, Z. H., Nakhla, T., Shabanova, V., Pyon, K., Bhat, V., & Giri, J. (2026).

Transcutaneous bilirubin measurements preterm infants: The impact of race, age, ar phototherapy. Journal of Perinatolog https://doi.org/10.1038/s41372-025-02558-w

4. Dam-Vervloet, A. J., Morsink, C. F., Krommendij M. E., Nijholt, I. M., van Straaten, H. L. M., Poc L., & Bosschaart, N. (2025). Skin color influence transcutaneous bilirubin measurements: systematic in vitro evaluation. Pediatric Researcl 97(4), 1706–1710. https://doi.org/10.1038/s41390-024-03081-y
5. Hansen, T. W. R., Nietsch, L., Norman, E., Bjerre, J. V., Hascoet, J. M., Mreihil, K., Ebbesen, F., & the Phototherapy Study Group. (2021). Reversibility of acute intermediate phase bilirubin encephalopathy. Acta Paediatrica, 110(1), 253– 259. https://doi.org/10.1111/apa.15367
6. Kaplan, M., Hammerman, C., & Maisels, M. (2011). Bilirubin genetics for the nongeneticis Hereditary defects of neonatal bilirubin conjugation. Pediatrics, 127(2), 364–373.
7. Kuzniewicz, M. W., Wickremasinghe, A. C., Wu, Y. W., McCulloch, C. E., Walsh, E. M., & Newman, T. B. (2014). Incidence, etiology, and outcomes of hazardous hyperbilirubinemia in newborns. Pediatrics, 134(3), 504–509. https://doi.org/10.1542/peds.2014-0987
8. Lazanovich, V. A., & Prosekova, E. V. (2014 Intravenous immunoglobulins: Mechanisms therapeutic effects. Medical Immunology, 16(4 311–322.
9. Maisels, M. J., & Kring, E. (2008). Transcutaneou bilirubinometry decreases the need for seru bilirubin measurements and saves mone Pediatrics, 117(1), 176–178.
10. Maisels, M. J., & McDonagh, A. F. (2008). Phototherapy for neonatal jaundice. New England Journal of Medicine, 358(9), 920–928. https://doi.org/10.1056/NEJMct0708376
11. McDonagh, A. F., & Lightner, D. A. (1985). 'Like a shrivelled blood orange'--bilirubin, jaundice, and phototherapy. Pediatrics, 75(3), 443–455.
12. Moiseenko, R. O., Pedan, V. B., & Vein, N. Y. (2006). Clinical protocol for providing neonatological care to children (Order No. 255 of the Ministry of Health of Ukraine). Kiev.
13. Nikonov, N. B., Nikonova, L. A., & Nikonova, F. N. (2019). The influence of newborn jaundice on the occurrence of complications in the form of cerebral palsy. Medicine. Sociology. Philosophy. Applied Research, 3, 45–51.
14. Olusanya, B. O., Kaplan, M., & Hansen, T. W. R. (2018). Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation: An update with clarifications. Pediatrics, 142(3), e20181815. https://doi.org/10.1542/peds.2018-1815
15. Prokopenko, T. V., Minguyova, L. K., & Smakovskaya, T. F. (2007). Significant factors in the development of hyperbilirubinemia in newborns. In Proceedings of the 4th Congress of Pediatricians of Russia (p. 554).
16. Sgro, M., Campbell, D. M., Kandasamy, S., & Shah, V. (2020). Variation in the use of phototherapy among neonatal intensive care units in Canada. Paediatrics & Child Health, 25(2), 96–101. https://doi.org/10.1093/pch/pxz046
17. Slusher, T. M., Olusanya, B. O., Vreman, H. J., Brearley, A. M., Vaucher, Y. E., Lund, T. C., Wong, R. J., Emokpae, A. A., & Stevenson, D. K. (2015). A randomized trial of phototherapy with filtered sunlight in African neonates. New England Journal of Medicine, 373(12), 1115–1124. https://doi.org/10.1056/NEJMoa1501074
18. Yatsyk, G. V., Belyaeva, I. A., & Odinaeva, N. D. (2008). Neonatal jaundice. Adamant.

Table 1. *Demographic Characteristics of the Study Participants (n=243)*

Group	Subgroup	n (%)
0–1 month	**Total**	**150 (61.7%)**
	Full-term	85 (35.0%)
	Preterm	65 (26.7%)
	• 2000-2500 g	*28 (11.5%)*
	• 1500-1999 g	*22 (9.1%)*
	• < 1500 g	*15 (6.2%)*
1–12 months	**Total**	**93 (38.3%)**
	Full-term	55 (22.6%)
	Preterm	38 (15.6%)

Table 2.1. *Bilirubin levels in subgroups with complete data variability*

Group	Subgroup	n	Bilirubin before (µmol/L) mean ± SD	Bilirubin after (µmol/L) mean ± SD	Statistical analysis
0–1 month	Full-term	85	195.00 ± 12.73	134.00 ± 14.14	Paired t-test: p = 0.010[1]
0–1 month	Preterm 2000–2500 g	28	207.50 ± 10.61	90.00 ± 7.07	Paired t-test: p = 0.002[2]
1–12 months	Full-term	55	199.50 ± 6.36	90.00 ± 7.07	Paired t-test: p = 0.003[2]
1–12 months	Preterm	38	195.00 ± 7.07	82.50 ± 3.54	Paired t-test: p = 0.014[1]

[1] After Bonferroni correction for 10 subgroup comparisons (adjusted α = 0.005), this p-value is considered nominally significant and exploratory.
[2] p-value remains statistically significant after Bonferroni correction.

Table 2.2. *Bilirubin levels in subgroups with protocol-driven uniform post-treatment values*

Group	Subgroup	n	**Bilirubin before (µmol/L)** median (IQR)	**Bilirubin after (µmol/L)** median (IQR)	Statistical analysis
0–1 month	Preterm 1500–1999 g	22	137.50 (115.0–160.0)	80.0 (80.0–80.0)[3]	Wilcoxon signed-rank test: p < 0.001[4]
0–1 month	Preterm <1500 g	15	105.0 (105.0–105.0)	70.0 (70.0–70.0)[3]	Wilcoxon signed-rank test: p < 0.001[4]

[3] Uniform post-treatment values reflect the clinical protocol: phototherapy was continued until the bilirubin level reached a predefined safety threshold (80 µmol/L for 1500–1999 g; 70 µmol/L for <1500 g), at which point treatment was stopped.
[4] Because all post-treatment values are identical, the paired t-test is not applicable; the Wilcoxon signed-rank test confirms a significant reduction (p < 0.001) despite the absence of post-treatment variability.

Note on correlation: For the overall cohort (n = 486 paired measurements), the correlation between transcutaneous (TcB) and total serum bilirubin (TSB) was r = 0.94 (p < 0.001), supporting the general utility of TcB as a monitoring tool. However, correlation is not reported separately for the subgroups in Table 2.2, because a valid correlation coefficient cannot be calculated when one variable has zero variance.

PERIÓDICO TCHÊ QUÍMICA
LETTERS TO THE EDITOR

CONTAMINANTES INVISÍVEIS, IMPACTOS VISÍVEIS: INTEGRANDO A QUÍMICA AMBIENTAL ÀS ESTRATÉGIAS DE CONSERVAÇÃO DA AMAZÔNIA

INVISIBLE CONTAMINANTS, VISIBLE IMPACTS: INTEGRATING ENVIRONMENTAL CHEMISTRY INTO AMAZON CONSERVATION STRATEGIES

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RESUMO

A poluição química é um importante, porém subestimado, fator de perda de biodiversidade na Amazônia, onde efluentes urbanos, pesticidas, metais e hidrocarbonetos interagem com as condições ambientais regionais para ameaçar os ecossistemas de água doce e terrestres. A rápida urbanização, o tratamento insuficiente de efluentes, a expansão agrícola e as atividades de extração de petróleo e mineração intensificam os impactos de misturas complexas de contaminantes. Os efluentes urbanos constituem uma das principais vias de contaminação, com estudos detectando dezenas de fármacos e contaminantes emergentes coexistindo nos rios, frequentemente em altas concentrações nas proximidades de grandes cidades, impondo riscos crônicos que podem afetar uma grande proporção das espécies aquáticas. Os pesticidas agravam ainda mais essas pressões, com múltiplos compostos frequentemente identificados em áreas urbanas e agrícolas, por vezes em níveis associados a riscos ecológicos moderados a elevados para invertebrados e peixes, enquanto a conectividade hidrológica facilita seu transporte generalizado e a exposição a misturas. Além disso, metais e hidrocarbonetos policíclicos aromáticos contribuem para o estresse ambiental cumulativo, com evidências de riscos ecológicos e potenciais efeitos biológicos de longo prazo, mesmo na ausência de toxicidade aguda. Em conjunto, esses achados ressaltam a necessidade de integrar a química ambiental às estratégias de conservação da Amazônia por meio de monitoramento em escala de bacia hidrográfica, inclusão de contaminantes emergentes nos marcos regulatórios e alinhamento entre avaliações de biodiversidade e avaliações químicas. Reconhecer a poluição química como um fator central da perda de biodiversidade é essencial para subsidiar políticas eficazes e prevenir a degradação adicional dos ecossistemas amazônicos e dos serviços que eles prestam.

Palavras-chave: *Poluição química, pesticidas, biodiversidade, contaminantes emergentes.*

ABSTRACT

Chemical pollution is an important yet underrepresented driver of biodiversity loss in the Amazon, where urban wastewater, pesticides, metals, and hydrocarbons interact with regional environmental conditions to threaten freshwater and terrestrial ecosystems. Rapid urbanization, limited wastewater treatment, agricultural expansion, and oil and mining activities intensify the impacts of complex contaminant mixtures. Urban wastewater is a major pathway, with studies detecting dozens of pharmaceuticals and emerging contaminants co-occurring in rivers, often reaching high concentrations near major cities, posing chronic risks that may affect a large proportion of aquatic species. Pesticides further exacerbate these pressures, with multiple compounds frequently identified in urban and agricultural areas, sometimes at levels associated with moderate to high ecological risks to invertebrates and fish, while hydrological connectivity facilitates their widespread transport and mixture exposure. In addition, metals and polycyclic aromatic hydrocarbons contribute to cumulative environmental stress, with evidence of ecological risks and potential long-term biological effects even in the absence of acute toxicity. Collectively, these findings underscore the need to integrate environmental chemistry into Amazon conservation strategies through basin-wide monitoring, inclusion of emerging contaminants in regulatory frameworks, and alignment of biodiversity and chemical assessments. Recognizing chemical pollution as a central driver of biodiversity loss is essential to support effective policies and prevent further degradation of Amazonian ecosystems and their services.

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Keywords: *Chemical pollution, pesticides, biodiversity, emerging contaminants.*

Dear Editor,

Chemical pollution plays a significant role in the decline of biodiversity in the Amazon, yet it remains an underrepresented factor. Despite increasing evidence, pollutants from urban wastewater, pesticides, metals, and petroleum hydrocarbons interact with local environmental conditions, posing threats to both freshwater and terrestrial ecosystems (Rico *et al.*, 2021; Sigmund *et al.*, 2023; Ojija, 2024). The rapid pace of urban growth, inadequate wastewater treatment, the expansion of agricultural areas, and activities related to oil and mining contribute to a complex array of stressors that intensify the ecological effects of these chemical mixtures (Rico *et al.,* 2021; Cabrera *et al.*, 2023; Rizzi *et al.,* 2023; Guarda *et al.,* 2020).

Urban wastewater serves as a significant conduit. Research conducted along the Amazon River and its primary tributaries, involving the monitoring of 43 pharmaceuticals and other urban pollutants at 40 locations, revealed combinations of up to 40 different substances, with some reaching global peak concentrations near urban centers like Manaus, Santarém, Macapá, and Belém (Rico *et al.*, 2021). Analysis of species sensitivity distribution suggests that these combinations could have prolonged impacts on 50-80% of aquatic species in proximity to urban areas, indicating that pollution hotspots in cities likely play a role in the decline of freshwater biodiversity (Rico *et al.*, 2021). An additional comprehensive screening (target + suspect LC HRMS) detected 51 pharmaceuticals, illegal drugs, and metabolites within the same river system, with 30 – 40 compounds coexisting in smaller urban tributaries and widespread markers (e.g., caffeine, cotinine, cocaine) even in regions with seemingly minimal human influence (Fabregat-Safont *et al.*, 2021). These observations align with global analyses showing that emerging contaminants (such as pharmaceuticals, personal care products, endocrine disruptors, and other organic substances) are prevalent, frequently unregulated, and not effectively removed by standard wastewater treatment processes, posing ongoing risks to living organisms and ecosystem health (Puri *et al.*, 2023; Li *et al.*, 2024; Morin-Crini *et al.*, 2022; Boro *et al.,* 2025; Petrie *et al.,* 2015; Khan *et al.,* 2021; Rasheed *et al.,* 2019; Starling *et al.*, 2019).

Pesticides also contribute to altering Amazonian hydrology and land use, leading to biodiversity decline. In the urban waterways of Manaus, Santarém, Macapá, and Belém, researchers identified 18 pesticides and 5 transformation products, with samples containing up to 8 different compounds. Notably, malathion, carbendazim, and bulk chlorpyrifos were found at concentrations exceeding 100 ng L^{-1} (Rico *et al.*, 2021). Risk evaluations indicated that malathion, chlorpyrifos, and chlorpyrifos methyl pose moderate to high risks to freshwater invertebrates, while malathion presents a moderate risk to fish. Species sensitivity distributions suggested that 5–44% of invertebrate species might be impacted in certain urban and agriculturally influenced areas (Rico *et al.,* 2021). In the Napo basin of the Ecuadorian Amazon, residues from 27 pesticides were detected across all 40 sites, including protected zones. Mixtures of organophosphate insecticides and the neonicotinoid imidacloprid were estimated to potentially affect 26–29% of aquatic species, with the greatest risks in rivers draining areas of African oil palm and corn cultivation (Cabrera *et al.,* 2023). In the Tocantins region, clomazone and other active substances were consistently found in surface waters, reaching concentrations of 0.538 µg L^{-1}, posing risks of bioaccumulation and biomagnification for aquatic life and human communities (Guarda *et al.,* 2020). These findings demonstrate how intensive agriculture and urbanization, coupled with high rainfall and hydrological connectivity, promote the widespread transport of pesticides, exposure to mixtures, and community-level impacts in Amazonian waters (Rico *et al.*, 2021; Cabrera *et al.,* 2023; Guarda *et al.*, 2020).

Metals and hydrocarbons exert additional stress. Evaluations at the basin scale, although not included in the provided abstracts, align with regional trends and indicate that metals from mining, urban runoff, and waste can surpass guideline thresholds, leading to phytotoxicity and ecological risks in the rivers of the Andean Amazon (Ojija, 2024). Polycyclic aromatic hydrocarbons (PAHs), linked to combustion and oil-related activities, are now prevalent in the surface waters of the Amazon: 16 priority PAHs have been detected at concentrations of 134 ng L^{-1} in the main river and 163 ng L^{-1} near heavily populated areas, with high molecular weight, pyrogenic PAHs being dominant and petrogenic signatures found near urban and oil-affected

zones (Rizzi *et al.,* 2023). Although current PAH concentrations are not anticipated to cause immediate toxicity, the authors advise ongoing monitoring near urban centers due to potential chronic and combined effects (Rizzi *et al*., 2023). These observations support global reviews that indicate hydrocarbons and other emerging contaminants can disrupt endocrine systems, alter genetic material, and diminish wildlife resilience, thereby threatening biodiversity and ecosystem services (Li *et al*., 2024; Ojija, 2024; Morin-Crini *et al.,* 2022; Khan *et al.,* 2021; Kasonga *et al.,* 2020; Rasheed *et al.,* 2019).

This body of evidence underscores the importance of thoroughly integrating environmental chemistry into conservation and monitoring strategies for the Amazon. Firstly, it is essential to establish routine, comprehensive chemical monitoring across the basin, employing wide-scope LC HRMS, suspect/non-target screening, and mixture toxicity tools like species sensitivity distributions, as demonstrated in the Amazon River (Rico *et al*., 2021; Cabrera *et al*., 2023; Petrie *et al*., 2015; Rizzi *et al*., 2023; Fabregat-Safont *et al*., 2021). These techniques can detect widespread indicators of human impact, such as specific pharmaceuticals or nicotine metabolites, prioritize high-risk pesticides and urban pollutants, and directly connect chemical profiles to ecologically significant outcomes (Rico *et al.,* 2021; Cabrera *et al*., 2023; Peter *et al*., 2022; Fabregat-Safont *et al*., 2021). Secondly, water quality regulations and river basin management plans in Amazonian countries should explicitly address emerging contaminants of concern — such as pharmaceuticals, personal care products, pesticides, and industrial organics — broadening the focus beyond traditional parameters, as advised by global policy and technical reviews (Puri *et al*., 2023; Morin Crini *et al*., 2022; Boro *et al*., 2025; Rasheed *et al*., 2019; Starling *et al*., 2019). Thirdly, biodiversity monitoring initiatives should be collaboratively designed with ecotoxicologists and environmental chemists to ensure that biological surveys and chemical assessments are conducted in the same locations and timeframes, facilitating the causal attribution of biodiversity changes to specific mixtures and stressor combinations (Rico *et al*., 2021; Sigmund *et al*., 2023; Rico *et al.,* 2021; Cabrera *et al*., 2023; Peter *et al*., 2022; Rizzi *et al*., 2023). Lastly, the emerging UN Science Policy Panel on Chemicals, Waste, and Pollution Prevention presents an opportunity to position Amazon pollution within the broader context of interconnected crises such as climate change, biodiversity loss, and chemical pollution, and to direct resources and guidance to low- and middle-income countries in the region (Sigmund *et al*., 2023; Wang *et al*., 2024; Diamond *et al.,* 2024).

To effectively tackle the Amazon biodiversity crisis, it is essential to acknowledge and address chemical pollution (such as wastewater contaminants, pesticides, metals, and hydrocarbons) as a primary factor rather than a secondary issue. Incorporating environmental chemistry tools systematically into monitoring, regulation, and conservation policies would establish the necessary evidence base to avert further, largely unseen degradation of Amazonian biodiversity and ecosystem services.

Sincerely,

Paulo Roberto Barros Gomes

5. DECLARATIONS

5.1. Study Limitations

As a letter to the editor, this text is inherently limited by the constraints typical of such publications, particularly in terms of analytical depth. The concise format restricts the ability to provide detailed methodological descriptions of the studies cited and limits a more comprehensive critical evaluation of the data. Additionally, the text predominantly relies on secondary data from previously published research, which, while it enhances the synthesis of existing knowledge, may limit empirical originality. The heterogeneity of the sources used, which include various methodological approaches and different spatial and temporal scales, complicates direct comparisons and the generalization of findings across the entire Amazon basin. Furthermore, potential spatial and sampling gaps should be considered, as many studies focus on urban areas or specific regions and do not uniformly represent the environmental diversity of the Amazon. There are also challenges in establishing causality, as much of the evidence is based on associations between contaminants and ecological effects, particularly in complex mixture exposure scenarios. Finally, the scarcity of long-term data constrains a more comprehensive understanding of the chronic and cumulative impacts of chemical pollution on biodiversity.

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5.3. Funding Sources

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5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw data are available upon request from the corresponding author.

5.6. Author Contributions (Contribuições dos Autores)

Specify the exact role of each author using the following standard codes: (Especifique o papel exato de cada autor usando os seguintes códigos padrão:)

Paulo Roberto Barros Gomes: CD, DC, DAI, MW, FA

Code	English	Português
CD	Conception and Design	Concepção e Design
DC	Data Collection	Coleta de Dados
DAI	Data Analysis and Interpretation	Análise e Interpretação de Dados
MW	Manuscript Writing	Escrita do Manuscrito
CR	Critical Review	Revisão Crítica
FA	Final Approval	Aprovação Final

5.7. AI and Computational Tools Declaration

1. Tool: ChatGPT, Consensus, and Grammarly. Purpose: Literature summarization, grammar/style improvement.
2. Human Verification: yes.

5.8. Research Integrity Declaration

Certify that your research meets the standards of research integrity. Check all that apply:

X - No data fabrication

X - No results falsification

X - No P-hacking or selective reporting

X - Original work (Trabalho original)

X - Not previously published

X - Methods conducted ethically

5.9. Originality & Plagiarism Statement

This manuscript is original and contains no plagiarism, as the similarity score was evaluated using Turnitin, which reported a similarity score of 20%.

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5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

Not applicable.

7. REFERENCES:

1. Boro, D., Chirania, M., Verma, A., Chettri, D., & Verma, A. (2025). Comprehensive approaches to managing emerging contaminants in wastewater: identification, sources, monitoring and remediation. *Environmental Monitoring and Assessment*, 197. https://doi.org/10.1007/s10661-025-13809-w.
2. Cabrera, M., Capparelli, M., Ñacato-Ch, C., Moulatlet, G., López-Heras, I., González, M., Alvear-S, D., & Rico, A. (2023). Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon.. *Chemosphere*, 139286. https://doi.org/10.1016/j.chemosphere.2023.139286.
3. Diamond, M., Sigmund, G., Bertram, M., Ford, A., Ågerstrand, M., Carlini, G., Lohmann, R., Sebková, K., Soehl, A., Starling, M., Suzuki, N., Venier, M., Vlahos, P., & Scheringer, M. (2024). Exploring Outputs of the Intergovernmental Science-Policy Panel on Chemicals, Waste, and Pollution Prevention. *Environmental Science & Technology Letters*. https://doi.org/10.1021/acs.estlett.4c00294.
4. Fabregat-Safont, D., Ibáñez, M., Bijlsma, L., Hernández, F., Waichman, A., De Oliveira, R., & Rico, A. (2021). Wide scope screening of pharmaceuticals, illicit drugs and their metabolites in the Amazon River.. *Water research*, 200, 117251. https://doi.org/10.1016/j.watres.2021.117251.
5. Guarda, P., Pontes, A., De S. Domiciano, R., Gualberto, L., Mendes, D., Guarda, E., & Da Silva, J. (2020). Assessment of Ecological Risk and Environmental Behavior of Pesticides in Environmental Compartments of the Formoso River in Tocantins, Brazil. *Archives of Environmental Contamination and Toxicology*, 79, 524 - 536. https://doi.org/10.1007/s00244-020-00770-7.
6. Kasonga, T., Coetzee, M., Kamika, I., Ngole-Jeme, V., & Momba, M. (2020). Endocrine-disruptive chemicals as contaminants of emerging concern in wastewater and surface water: A review.. *Journal of environmental management*, 277, 111485. https://doi.org/10.1016/j.jenvman.2020.111485.
7. Khan, S., Naushad, M., Govarthanan, M., Iqbal, J., & Alfadul, S. (2021). Emerging contaminants of high concern for the environment: Current trends and future research. *Environmental research*, 112609. https://doi.org/10.1016/j.envres.2021.112609.
8. Li, X., Shen, X., Jiang, W., Xi, Y., & Li, S. (2024). Comprehensive review of emerging contaminants: Detection technologies, environmental impact, and management strategies. *Ecotoxicology and environmental safety*, 278, 116420. https://doi.org/10.1016/j.ecoenv.2024.116420.
9. Morin-Crini, N., Lichtfouse, E., Liu, G., Balaram, V., Ribeiro, A., Lu, Z., Stock, F., Carmona, E., Teixeira, M., Picos Corrales, L., Moreno-Piraján, J., Giraldo, L., Li, C., Pandey, A., Hocquet, D., Torri, G., & Crini, G. (2022). Worldwide cases of water pollution by emerging contaminants: a review. *Environmental Chemistry Letters*, 20, 2311 - 2338. https://doi.org/10.1007/s10311-022-01447-4.
10. Ojija, F. (2024). Emerging environmental contaminants: Sources, effects on biodiversity and humans, remediation, and conservation implications. *Science Progress,* 107. https://doi.org/10.1177/00368504241253720.
11. Peter, K., Lundin, J., Wu, C., Feist, B., Tian, Z., Cameron, J., Scholz, N., & Kolodziej, E. (2022). Characterizing the Chemical Profile of Biological Decline in Stormwater-Impacted Urban Watersheds. *Environmental science & technology*. https://doi.org/10.1021/acs.est.1c08274.
12. Petrie, B., Barden, R., & Kasprzyk-Hordern, B. (2015). A review on emerging

contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water research*, 72, 3-27. https://doi.org/10.1016/j.watres.2014.08.053.

13. Puri, M., Gandhi, K., & Kumar, M. (2023). Emerging environmental contaminants: A global perspective on policies and regulations.. *Journal of environmental management*, 332, 117344. https://doi.org/10.1016/j.jenvman.2023.117344.
14. Rasheed, T., Bilal, M., Nabeel, F., Adeel, M., & Iqbal, H. (2019). Environmentally-related contaminants of high concern: Potential sources and analytical modalities for detection, quantification, and treatment.. *Environment international*, 122, 52-66. https://doi.org/10.1016/j.envint.2018.11.038.
15. Rico, A., De Oliveira, R., De Souza Nunes, G., Rizzi, C., Villa, S., López-Heras, I., Vighi, M., & Waichman, A. (2021). Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems.. *Environment international,* 155, 106702. https://doi.org/10.1016/j.envint.2021.106702
16. Rico, A., De Oliveira, R., De Souza Nunes, G., Rizzi, C., Villa, S., De Caroli Vizioli, B., Montagner, C., & Waichman, A. (2021). Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon.. *Chemosphere*, 132821. https://doi.org/10.1016/j.chemosphere.2021.132821.
17. Rizzi, C., Villa, S., Waichman, A., De Souza Nunes, G., De Oliveira, R., Vighi, M., & Rico, A. (2023). Occurrence, sources, and ecological risks of polycyclic aromatic hydrocarbons (PAHs) in the Amazon river.. *Chemosphere*, 139285. https://doi.org/10.1016/j.chemosphere.2023.139285.
18. Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M., Erdelen, W., Evers, D., Hoffmann, T., Hueffer, T., Lai, A., Torres, J., Mueller, L., Perrigo, A., Rillig, M., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., Soehl, A., Triebskorn, R., Vlahos, P., Berg, C., Wang, Z., & Groh, K. (2023). Addressing chemical pollution in biodiversity research. *Global Change Biology*, 29, 3240 - 3255. https://doi.org/10.1111/gcb.16689.
19. Starling, M., Amorim, C., & Leão, M. (2019). Occurrence, control and fate of contaminants of emerging concern in environmental compartments in Brazil.. *Journal of hazardous materials*, 372, 17-36. https://doi.org/10.1016/j.jhazmat.2018.04.04.
20. Wang, F., Xiang, L., Leung, K., Elsner, M., Zhang, Y., Guo, Y., Pan, B., Sun, H., An, T., Ying, G., Brooks, B., Hou, D., Helbling, D., Sun, J., Qiu, H., Vogel, T., Zhang, W., Gao, Y., Simpson, M., Luo, Y., Chang, S., Su, G., Wong, B., Fu, T., Zhu, D., Jobst, K., Ge, C., Coulon, F., Harindintwali, J., Zeng, X., Wang, H., Fu, Y., Wei, Z., Lohmann, R., Chen, C., Song, Y., Sanchez-Cid, C., Wang, Y., El-Naggar, A., Yao, Y., Huang, Y., Law, J., Gu, C., Shen, H., Gao, Y., Qin, C., Li, H., Zhang, T., Corcoll, N., Liu, M., Alessi, D., Li, H., Brandt, K., Picó, Y., Gu, C., Guo, J., Su, J., Corvini, P., Ye, M., Rocha-Santos, T., He, H., Yang, Y., Tong, M., Zhang, W., Suanon, F., Brahushi, F., Wang, Z., Hashsham, S., Virta, M., Yuan, Q., Jiang, G., Tremblay, L., Bu, Q., Wu, J., Peijnenburg, W., Topp, E., Cao, X., Jiang, X., Zheng, M., Zhang, T., Luo, Y., Zhu, L., Li, X., Barcelo, D., Chen, J., Xing, B., Amelung, W., Cai, Z., Naidu, R., Shen, Q., Pawliszyn, J., Zhu, Y., Schaeffer, A., Rillig, M., Wu, F., Yu, G., & Tiedje, J. (2024). Emerging contaminants: A One Health perspective. *The Innovation*, 5. https://doi.org/10.1016/j.xinn.2024.100612

PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

DETERMINAÇÃO DE AMINAS BIOGÊNICAS E NITRITO RESIDUAL EM EMBUTIDOS TRATADOS COM SORO DE LEITE LÍQUIDO E EM PÓ COM ÁCIDO ASCÓRBICO E ARMAZENADOS SOB REFRIGERAÇÃO

DETERMINATION OF BIOGENIC AMINES AND RESIDUAL NITRITE IN SAUSAGES TREATED WITH LIQUID AND DRY WHEY WITH ASCORBIC ACID UNDER REFRIGERATED STORAGE

تحديد الأمينات الحيوية والنتريت المتبقي في النقانق المعالجة بمصل اللبن السائل والجاف مع حمض الأسكوربيك تحت ظروف الخزن المبرد

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RESUMO

Introdução: Na indústria de carnes, nitritos e nitratos são utilizados em embutidos por suas propriedades bem conhecidas. No entanto, existem preocupações com a saúde devido ao seu potencial de formar compostos carcinogênicos, de modo que estudos estão em andamento para identificar alternativas naturais. **Objetivo**: A pesquisa examinou o impacto da incorporação de soro de leite líquido ácido e soro de leite em pó com ácido ascórbico sobre os níveis de aminas biogênicas e a medição de nitrito residual em embutidos recém-produzidos e refrigerados. **Métodos**: O estudo compreendeu dois experimentos. O primeiro experimento apresentou cinco tratamentos: T1- Nitrito de Sódio 0,005%; T2- Nitrito de Sódio 0,005% + Soro Líquido 5 ml; T3- Nitrito de Sódio 0,002% + Soro Líquido 5 ml; T4- Soro Líquido 5 ml; T5- Soro Líquido 5 ml + Ácido Ascórbico 0,05%. O segundo experimento englobou seis tratamentos: T1- Nitrito de Sódio 0,005%; T2- Nitrito de Sódio 0,005% + Soro em Pó 2%; T3- Nitrito de Sódio 0,005% + Soro em Pó 5%; T4- Nitrato de Sódio 0,002% + Soro em Pó 2%; T5- Soro em Pó 5%; T6- Soro em Pó 5% + Ácido Ascórbico 0,05%. Após o processo de fabricação e um período de armazenamento de 21 dias, os níveis de aminas biogênicas e de nitrito residual foram avaliados. **Resultados**: A incorporação tanto de soro de leite líquido ácido quanto de soro em pó, juntamente com ácido ascórbico, resultou em redução das aminas biogênicas nos embutidos. Diferenças significativas foram observadas entre o tratamento contendo exclusivamente nitrito de sódio e aqueles incorporando apenas soro de leite líquido ácido ou soro em pó, bem como aqueles com ácido ascórbico combinado com soro de leite líquido ácido ou soro em pó. A investigação também revelou uma diminuição nos níveis de nitrito residual em embutidos tratados com soro de leite líquido ácido e soro em pó, em conjunto com ácido ascórbico, em comparação com o tratamento que incluía apenas nitrito de sódio. **Discussão**: Esses aditivos resultaram em produtos cárneos processados com os menores níveis de aminas biogênicas e os menores valores de nitrito residual. Portanto, recomenda-se o uso de soro de leite tanto ácido quanto em pó, além de ácido ascórbico, em produtos cárneos processados como alternativa aos nitritos. **Conclusões**: O uso de soro de leite tanto líquido quanto em pó na produção de embutidos é uma estratégia inovadora para o desenvolvimento de produtos cárneos livres de nitrito, em consonância com a tendência de clean label (rótulo limpo).

Palavras-chave: *Soro de leite, ácido ascórbico, aminas biogênicas, nitrito residual, linguiça*.

ABSTRACT

Background: In the meat industry, nitrites and nitrates are used in sausages for their well-known properties. However, there are health concerns about their potential to form carcinogenic compounds, so studies are ongoing to identify natural alternatives. **Aim**: The research examined the impact of incorporating acidic liquid whey and dry whey with ascorbic acid on the levels of biogenic amines and the measurement of residual nitrite in freshly produced and refrigerated sausage. **Methods**: The study comprised two experiments: The first

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experiment featured five treatments: T1- Sodium Nitrite 0.005%; T2- Sodium Nitrite 0.005% + Liquid Whey 5 ml; T3- Sodium Nitrite 0.002% + Liquid Whey 5 ml; T4- Liquid Whey 5 ml; T5- Liquid Whey 5 ml + Ascorbic Acid 0.05%. The second experiment encompassed six treatments: T1- Sodium Nitrite 0.005%; T2- Sodium Nitrite 0.005% + Dry Whey 2%; T3- Sodium Nitrite 0.005% + Dry Whey 5%; T4- Sodium Nitrite 0.002% + Dry Whey 2%; T5- Dry Whey 5%; T6- Dry Whey 5% + Ascorbic Acid 0.05%. After the manufacturing process and a 21-day storage period, the levels of biogenic amines and residual nitrite were assessed. **Results**: The incorporation of both acidic liquid whey and dry whey, alongside ascorbic acid, resulted in a reduction of biogenic amines in the sausage. Significant differences were observed between the treatment containing solely sodium nitrite and those incorporating only acidic liquid whey or dry whey, as well as those with ascorbic acid combined with either acidic liquid whey or dry whey. The investigation also revealed a decrease in residual nitrite levels in sausages treated with acidic liquid whey and dry whey, in conjunction with ascorbic acid, compared to the treatment that included only sodium nitrite. **Discussion**: These additives resulted in processed meat products with the lowest levels of biogenic amines and the lowest residual nitrite values. Therefore, the use of both acidic and dry whey, in addition to ascorbic acid, in processed meat products is recommended as an alternative to nitrites.**Conclusions**: The use of both liquid and dry whey in sausage production is an innovative strategy for developing nitrite-free meat products, in line with the "clean label" trend.

Keywords*: Whey, ascorbic acid, biogenic amines, residual nitrite, sausage.*

الملخص:

الخلفية: تُستخدم النيتريتات والنترات في صناعة اللحوم، ولا سيما في إنتاج النقانق، نظرًا لخصائصها المعروفة. ومع ذلك، تثار مخاوف صحية بشأن قدرتها المحتملة على تكوين مركبات مسرطنة، مما دفع إلى إجراء دراسات مستمرة للبحث عن بدائل طبيعية. **الهدف:** هدفت هذه الدراسة إلى تقصّي تأثير إضافة مصل اللبن السائل الحمضي ومصل اللبن الجاف، مع حمض الأسكوربيك، في مستويات الأمينات الحيوية وقياس النتريت المتبقي في النقانق الطازجة والمخزنة تحت التبريد. **طرائق العمل:** اشتملت الدراسة على تجربتين: التجربة الأولى تضمّنت خمس معاملات: T1- نتريت الصوديوم 0.005%؛ T2- نتريت الصوديوم 0.005% + مصل اللبن السائل 5 مل؛ T3- نتريت الصوديوم 0.002% + مصل اللبن السائل 5 مل؛ T4- مصل اللبن السائل 5 مل؛ T5- مصل اللبن السائل 5 مل + حمض الأسكوربيك 0.05%. أما التجربة الثانية فقد شملت ست معاملات: T1- نتريت الصوديوم 0.005%؛ T2- نتريت الصوديوم 0.005% + مصل اللبن الجاف 2%؛ T3- نتريت الصوديوم 0.005% + مصل اللبن الجاف 5%؛ T4- نتريت الصوديوم 0.002% + مصل اللبن الجاف 2%؛ T5- مصل اللبن الجاف 5%؛ T6- مصل اللبن الجاف 5% + حمض الأسكوربيك 0.05%. وبعد عملية التصنيع وفترة خزن بلغت 21 يومًا، جرى تقييم مستويات الأمينات الحيوية والنتريت المتبقي. **النتائج:** أدّت إضافة كلٍّ من مصل اللبن السائل الحمضي ومصل اللبن الجاف، إلى جانب حمض الأسكوربيك، إلى خفض مستويات الأمينات الحيوية في النقانق. كما لوحظت فروق معنوية بين المعاملة التي احتوت على نتريت الصوديوم فقط وتلك التي احتوت على مصل اللبن السائل الحمضي أو مصل اللبن الجاف، فضلًا عن المعاملات التي جمعت بين حمض الأسكوربيك وأحد هذين النوعين من مصل اللبن. كما أظهرت النتائج انخفاضًا في مستويات النتريت المتبقي في النقانق المعالجة بمصل اللبن السائل الحمضي ومصل اللبن الجاف مع حمض الأسكوربيك، مقارنةً بالمعاملة التي تضمنت نتريت الصوديوم فقط. **المناقشة:** أسهمت هذه الإضافات في إنتاج منتجات لحوم مصنّعة تحتوي على أقل مستويات من الأمينات الحيوية وأدنى قيم للنتريت المتبقي. وبناءً على ذلك، يُوصى باستخدام كلٍّ من مصل اللبن السائل والجاف، إلى جانب حمض الأسكوربيك، في منتجات اللحوم المصنّعة بوصفها بديلًا عن النيتريتات. **الاستنتاجات:** يُعدّ استخدام كلٍّ من مصل اللبن السائل والجاف في إنتاج النقانق استراتيجية مبتكرة لتطوير منتجات لحوم خالية من النيتريت، بما يتماشى مع اتجاه "الملصق النظيف".

الكلمات المفتاحية: مصل اللبن، حمض الاسكوبيك، الامينات الحيوية، النتريت المتبقي، النقانق.

1. INTRODUCTION:

Sausage is regarded as one of the most ancient food products created by humans. Various variants of these items exist worldwide, influenced by the meat used, its varieties, the climatic conditions of the respective countries, cultural and religious traditions, and the processing methods employed (Carballo, 2021). Fermented sausage is regarded as the most sought-after meat product by consumers due to its superior nutritional content (Holck *et al.,* 2017). However, the processed meat company receives pressure from customers and nutritionists to enhance the nutritional value of its products. These standards entail eliminating detrimental additives to adhere to the "clean label" trend (Cegiełka, 2020) and the endeavor to reduce or eliminate the use of nitrites or nitrates in processing (Karwowska *et al*., 2021). Simultaneously, the omission of nitrates and nitrites from processed meat products poses technological challenges, as these additives play key roles in these products. Nitrites and nitrates hinder bacterial growth, particularly that of *Clostridium botulinum*, so restricting the oxidation process while still imparting the desirable color and flavor (Ma *et al*., 2018).

Owing to the versatility of nitrite and nitrate, numerous researchers are exploring viable alternatives to nitrite and nitrate salts for use in meat product processing (Alahakoon *et al*., 2015; Siekmann *et al*., 2021). One solution is the use of whey, a by-product of several dairy products, including yogurt, fresh and soft cheeses, and cream cheeses (Wherry *et al*., 2019). Whey typically comprises around 55% of the nutrients found in milk (Guimarães et al., 2010; Roshanghias and Madadlou, 2018).

The serum phase of milk is the liquid that remains after the removal of fat and casein, predominantly consisting of soluble components such as lactose, soluble salts, and globular proteins (Guimarães *et al.,* 2010; Chandraqaoala *et al*., 2016). Whey is a by-product generated during the preparation of acid-curdled cheeses, including cottage cheese, ricotta cheese, and Greek yogurt. This is due to increased production of Greek yogurt and cottage cheese.

The dairy industry faces mounting pressure to establish sustainable practices for recycling acid whey (Zotta *et al*., 2020). A study investigated the potential of acid whey as a substitute for nitrite or nitrate salts in fermented beef products (Alahakoon *et al.,* 2015; Kononiuk and Karwowska, 2020). A study indicated that using whey in the manufacture of dry-fermented sausages enhances their quality (Balev *et al*., 2005). Karageorgou (2023) demonstrated that adding whey to meat has significant benefits, as the quality characteristics of raw and cooked meat samples were evaluated, with results showing a marked improvement. A study by Li *et al*. (2025) indicated that incorporating whey into dried camel meat is a successful method for improving the flavor and quality of camel meat products. Its addition improved the characteristics of fermented dried camel meat.

In a similar context, researchers in a separate study utilized acid whey in conjunction with sodium ascorbate. The study's results indicated that incorporating sodium ascorbate influenced the processes involved in the production of unprocessed dry-fermented sausages. It was noted that it reduces lipid oxidation in meat products (Balev *et al*., 2005).
Fermented meat products represent a prevalent dietary source of biogenic amines (BAs). While these compounds can be found in various meat products, the production of fermented meats creates optimal environmental conditions that facilitate their accumulation due to the activity of microorganisms with decarboxylase activity. (Balev *et al.,*2005 , Schirone *et al*., 2022). These compounds serve as indicators of product quality and pose serious risks to public health. The most prevalent biogenic amines in fermented foods include Tyramine, putrescine, and cadaverine. Histamine is regarded as the most hazardous to human health, as it can induce a range of adverse effects known as "histamine poisoning" (Landete *et al*., 2008; Karwowska *et al*., 2021). Despite previous studies investigating natural alternatives to nitrite, limited research has focused on the combined use of liquid and dry whey with ascorbic acid and their effects on both biogenic amines and residual nitrite in fermented sausages. Therefore, this study aims to fill this gap.

This study aims to assess the impact of incorporating both liquid and dry whey with Ascorbic acid at different concentrations on the biogenic amine content in processed sausage stored under refrigeration, and to estimate residual nitrite levels.

2. MATERIALS AND METHODS:

Two studies were performed in the meat laboratory of the Animal Production Department at the College of Agriculture, University of Basra. The first experiment utilized liquid whey, while the second employed dried whey.

2.1. Materials

The materials used in this research were liquid and dried whey, beef, fat, starch, salt, spices, natural large intestine linings (sheep casings), ascorbic acid, and sodium nitrite. The equipment used included a spectrophotometer (Carbolit/England), a high-performance liquid chromatograph (HPLC) (Skyam/Germany), an oven (Memert/Germany), a sensitive balance (Denver/Germany), an electronic precision balance (Cittern/China), and an electric grinder (Moulines/France).

2.2 Methods:

2.2.1. Liquid and dry whey

Liquid whey was obtained from fresh cow's milk using the procedure outlined by Cipolat-Gotet *et al.* (2013), which entails heating the milk to 60 °C for 30 minutes. Subsequently, 5 cc of white vinegar was incorporated. After reducing the milk temperature to 45 °C, the milk was incubated at ambient laboratory conditions for 3 hours to complete the curdling process. The whey was removed from the cheese using a sterile cloth, and the whey arising from the milk coagulation was collected in a clean glass container.

2.2.2. Manufacturing of Sausages

The pure meat and fat were weighed in an 80:15 ratio, then minced using an electric mincer. Salt (2%), spices (1%), and starch (5%) were added to the pure meat and fat mixture, which was then hand-mixed to achieve initial

homogeneity. The mixture was then divided into five parts in the first experiment, where five treatments were prepared with different additives, namely T1- Sodium Nitrite 0.005%; T2- Sodium Nitrite 0.005% + Liquid Whey 5 ml; T3- Sodium Nitrite 0.002% + Liquid Whey 5 ml; T4- Liquid Whey 5 ml; and T5- Liquid Whey 5% + Ascorbic Acid 0.05%(Table 1). In the second experiment, six treatments were formulated with varying additions: T1 -Sodium Nitrite 0.005%; T2 -Sodium Nitrite 0.005% + Dry Whey 2%; T3 - Sodium Nitrite 0.005% + Dry Whey 5%; T4 - Sodium Nitrite 0.002% + Dry Whey 2%; T5 -Dry Whey 5%; T6 - Dry Whey 5% + Ascorbic Acid 0.05%). As seen in Table 2.

The mixture was then distributed into natural intestines, with each treatment weighing 1 kg and each sausage sample weighing 150 g. After processing, the treatments were stored at 4°C, and the analyses were performed after 21 days.

2.2.3. Estimation of residual nitrite

Residual nitrite was estimated at the Ministry of Science and Technology, Scientific Research Authority, Center for Environment, Water and Renewable Energy, Pollutant Treatment Department. The test was conducted according to Standard Specification No. 86 of 2014, and absorption was measured using a UV-Vis spectrophotometer at 538 nm. Results were expressed as ppm (mg/kg).

2.2.4. Estimation of biogenic amines

Biogenic amines were estimated at the Ministry of Science and Technology, Scientific Research Authority, Center for Environment, Water and Renewable Energy, Pollutant Treatment Department, using the method described by Weremf *et al*. (2020). The analysis was performed using high-performance liquid chromatography (HPLC) equipped with a UV detector, employing a C18 column (250mm × 4.6mm) as the stationary phase. The mobile phase consisted of acetonitrile and distilled water (5:5, v/v), delivered at a flow rate of 1.5 mL/min under a system pressure of 400-600 bar. The content of the biogenic amines was measured with Standard reference compounds: Methylamine, Phenylethylamine, Putrescine, Cadaverine, Histamine, Tyramine, Trimethylamine, and Spermidine (Figure 1, Table 3). Results were expressed as ppm (mg/kg).

2.2.5. Statistical Analysis and Mathematical Model

The experiment was conducted using a completely randomized design (CRD) with three independent replicates per treatment. Each measurement was performed in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments. Prior to analysis, data normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene tests, respectively. When significant differences were observed, means were compared using the Least Significant Difference (LSD) test at the $p \leq 0.01$ significance level.
All statistical analyses were conducted using GenStat software (version 12, VSN International Ltd., Hemel Hempstead, UK).

3. RESULTS AND DISCUSSION

3.1. Results

Table 4 presents the levels of biogenic amines in sausages treated with liquid whey and ascorbic acid after a 21-day aging period. Eight biogenic amines were detected at varying concentrations across the treatments: Methylamine, Phenylethylamine, Putrescine, Cadaverine, Histamine, Tyramine, Trimethylamine, and Spermidine. The results indicated significant differences at the probability level ($p \leq 0.01$) among the treatments regarding the concentration of biogenic amines. Specifically, the treatments incorporating liquid whey and ascorbic acid exhibited reduced or negligible levels of biogenic amines compared with the control treatment T1, which contained only 0.005% sodium nitrite. The biogenic amine methylamine exhibited a high concentration in T1 and T2, reaching 4.85 ppm in both treatments, whereas it decreased in T3 and T4 to 4.73 ppm and 4.72 ppm, respectively, and was not detected in T5. Phenylethylamine and Spermidine were detected exclusively in treatments T1 and T2, with phenylethylamine levels measuring 4.62 ppm and 4.88 ppm, respectively, while Spermidine was recorded at 5.01 ppm in both treatments. The highest concentration of putrescine was observed in T3 at 5.05 ppm, whereas T1, T2, and T4 recorded levels of 4.98 ppm, 4.98 ppm, and 4.70 ppm, respectively; T5 was not detected. Cadaverine was detected in all treatments at varying concentrations, with the greatest level measured at 5.22 ppm in T1 and the lowest at 4.90 ppm in T2. Histamine and

Trimethylamine were detected in all treatments at varying concentrations, with significant differences observed across treatments at the 0.01 level ($p < 0.01$).The findings indicated that Tyramine was present in treatments T1, T3, and T5, but absent in treatments T2 and T4.

Table 5 presents the biogenic amine levels in the sausage subjected to various proportions of dry whey and ascorbic acid after a 21-day aging period. Eight biogenic amines were observed at varying concentrations across treatments. The results showed significant variations at the probability level ($p < 0.01$) across treatments regarding the quantity of biogenic amines. The findings indicated that the sausage treated with dry whey and ascorbic acid exhibited reduced or negligible levels of biogenic amines compared with the control treatment T1, which contained only 0.005% sodium nitrite.

The results showed that methylamine was present in all treatments, except for treatment T5. Table 5 shows that phenylethylamine was detected in all treatments (T1, T2, T3, T4, and T5), with values of 4.62, 4.91, 4.98, 4.67, and 4.98 ppm, respectively, and was not detected in treatment T6. Putrescine was recorded in treatments T1, T2, T3, and T4 and ranged between 4.92ppm and 5.01ppm, and no appearance was recorded in treatments T5 and T6. The results also showed that cadaverine was present in T1, T2, and T3, with concentrations of 5.22, 5.85, and 4.96 ppm, respectively, and was not detected in the remaining treatments. Histamine was also detected in T1, T2, T3, T4, and T5, ranging from 5.04 ppm to 5.28 ppm, but not in T6. Tyramine was detected at 5.01, 5.00, and 5.06 ppm in T1, T2, and T4, respectively, and was not detected in the remaining treatments. The results showed that Trimethylamine was present in treatments T1, T2, T4, and T5, at levels ranging from 5.01 ppm to 5.17 ppm, and absent in the remaining treatments. The biogenic amine, Spermidine, was detected in only two treatments, T1 (5.01 ppm) and T4 (4.97 ppm), and was not detected in the remaining treatments.

Table 6 shows the amount of residual nitrite in the sausage treated with liquid whey and ascorbic acid after 21 days, where the results showed significant differences at the probability level ($p \leq 0.01$). All treatments exhibited residual nitrite in various concentrations, with T1 recording the highest value of 2.34 ppm and T5 the lowest at 0.71 ppm for residual nitrite. In T2, T3, and T4, the residual nitrite concentrations were 1.31, 1.13, and 1.81 ppm, respectively.

Table 7 shows the residual nitrite levels in sausage treated with dry whey and ascorbic acid after 21 days. The results showed significant differences at the 0.01 level of significance ($p \leq 0.01$). The highest residual nitrite value was observed in T1 at 2.34 ppm, whereas the lowest was observed in T6 at 0.71 ppm. The remaining treatments exhibited values ranging from 0.73 ppm to 1.71 ppm, with residual nitrite values in T2, T3, T4, and T5 recorded at 1.41, 1.71, 1.31, and 0.73 ppm, respectively.

3.2. Discussion

Meat products are among the most common dietary sources of biogenic amines. During sausage production, favorable environmental conditions favor the accumulation of these compounds through microbial activity (Schirone *et al*., 2022). Biogenic amines are important indicators of product quality and also have implications for public health (Latorre-Moratalla *et al*., 2012). The most common biogenic amines in meat products are Tyramine and cadaverine (Vasconcelos *et al*., 2021). In the current study, in addition to the aforementioned amines, the following were also identified: Methylamine, Phenylethylamine, Histamine, Trimethylamine, and Spermine. The results indicate that biogenic amines decreased in the majority of treatments using liquid whey, as well as in the treatment combining liquid whey with ascorbic acid, with some treatments showing no detectable levels. The inhibitory effect of acidic conditions on decarboxylase-positive microorganisms can explain the observed reduction in biogenic amines in whey-treated samples. In addition, whey promotes the growth of lactic acid bacteria, which compete with spoilage microorganisms and reduce amine formation. This mechanism is consistent with previous studies by Durak-Dados et al. (2020) and Latorre-Moratalla *et al*. (2017), who quantified biogenic amines in fermented dry sausages available in Spanish retail outlets. The results of the study were consistent with those of Karwowska *et al.* (2022), who used acidic liquid whey and ascorbic acid in fermented sausage.

The study also showed that treatment T6 did not show any noticeable concentrations of

biogenic amines, except for the appearance of methylamine. This means that the presence of dry whey with ascorbic acid has a significant effect on the decrease or absence of the appearance of biogenic amines after 21 days of fermentation. The reason may be attributed to the significant effect on the activity of microscopic organisms, as evidenced by the decarboxylase enzyme. This advantage is due to the acidic medium of the sausage treated with dry whey and ascorbic acid, which encourages the growth of beneficial lactic acid bacteria and reduces the activity of other non-beneficial microorganisms. These findings are consistent with those of Karwowska et al. (2022), who reported that whey reduces biogenic amine formation by lowering microbial decarboxylase activity. In this study, the histamine levels in the fermented sausage did not exceed the recommended limits. This is a good sign, as histamine is considered the most dangerous to human health because it causes various harmful effects, such as "histamine poisoning ". The European Food Safety Authority report has specified a recommended daily intake of histamine of less than 50 ppm for healthy individuals, but even lower for those with histamine intolerance.

As the study results demonstrated a reduction in residual nitrite levels across all treatments using liquid whey and ascorbic acid after 21 days, compared with T1. The reduction can be attributed to oxidation processes during storage, as corroborated by Tahmouzi et al. (2025) and Honik (2008).

The study showed that the treatments incorporating dry whey (T5) and dry whey with ascorbic acid (T6) exhibited the lowest residual nitrite levels after 21 days post-fermentation, attributable to the absence of nitrite in their composition. These diminished nitrite levels are a result of the meat and other constituents utilized in the sausage production process. This aligns with Zhang *at al.* (2023), who observed residual nitrite levels in samples not treated with sodium nitrite, attributing this to the nitrite content of the meat used in production. The reduction in residual nitrite may be attributed to ascorbic acid's antioxidant activity, which accelerates nitrite depletion.

4. CONCLUSIONS

The use of both liquid and dry whey in sausage production is an innovative strategy for developing nitrite-free meat products, in line with the "clean label" trend. The results indicate a positive effect of adding dry and liquid whey, along with ascorbic acid, on the quality of processed sausage. These additives resulted in processed meat products with the lowest levels of biogenic amines and the lowest residual nitrite values. Therefore, the use of both acidic and dry liquid whey, in addition to ascorbic acid, in processed meat products is recommended as an alternative to nitrites. However, further studies including microbiological, sensory, and physicochemical analyses are required to confirm its full applicability.

These findings highlight the potential of whey as a sustainable and natural alternative to nitrite in processed meat products, supporting clean-label production strategies.

5. DECLARATIONS

5.1. Study Limitations

The sample size was limited to laboratory-scale experiments, which may not fully represent industrial conditions. Second, microbial analysis was not conducted to confirm the inhibition of decarboxylase-positive bacteria directly. Third, the storage period was limited to 21 days, and the effects of longer storage were not evaluated. Future studies should include microbial profiling and extended storage analysis to validate these findings.

5.2. Acknowledgments

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5.3. Funding Sources

No external funding was received. The authors have covered all publication expenses.

5.4. Conflicts of Interest

The authors declare no conflicts of interest.

5.5. Data Availability

All data presented in this study are available in the manuscript tables. Raw data are available upon request from the corresponding author.

5.6. Author Contributions

Noor Falah Mahdi Al-Kenane: CD, DC, DAI, MW, and FA.

Amera Kadhim Nasser: CD, CR, and FA.

5.7. AI and Computational Tools Declaration

Tool: Grammarly (Grammarly Inc.), QuillBot (QuillBot Inc.), Turnitin (Turnitin LLC).
Purpose: Grammarly was used for grammar and language editing. QuillBot was used for limited paraphrasing in the introduction section.

Turnitin was used for plagiarism detection and similarity checking. Extent: The use of AI tools was limited to language improvement and minor text refinement (less than 10% of the manuscript).

Human Verification: All outputs generated or modified using AI tools were carefully reviewed, validated, and substantially revised by the authors. No AI tools were used for data analysis, statistical testing, results interpretation, or scientific decision-making.

5.8. Research Integrity Declaration

The authors certify that this research adheres to the highest standards of research integrity. Specifically, we confirm that no data fabrication or falsification was performed; no p-hacking or selective reporting of results was conducted; this work is original and has not been published previously; and all methods were conducted in accordance with relevant ethical guidelines and regulations.

5.9. Originality Statement

This manuscript is the original work of the authors. This manuscript is not under consideration for publication elsewhere.

5.10. Open Access

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

Ethics approval was not required for the present research.

6.2. Informed Consent

Not applicable.

7- REFERENCES

1- Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., and Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. *Trends in Food Science & Technology*, *45* (1), 37-49. DOI: https://doi.org/10.1016/j.tifs.2015.05.008
2- Balev, D., Vulkova, T., Dragoev, S., Zlatanov, M., and Bahtchevanska, S. (2005). A comparative study on the effect of some antioxidants on the lipid and pigment oxidation in dry-fermented sausages. *International Journal of Food Science and Technology*, *40*(9), 977-983. DOI: 10.1111/j.1365-2621.2005.01031.x
3- Carballo, J. (2021). Sausages: Nutrition, safety, processing and quality improvement. *Foods*, *10*(4), 890. DOI: https://doi.org/10.3390/foods10040890
4- Cegielka, A. (2020). "Clean label" as one of the leading trends in the meat industry in the world and in Poland: a review. *Roczniki*

Państwowego Zakładu Higieny, *71*(1). DOI: 10.32394/rpzh.2020.0098

5- Chandrapala, J., Duke, M. C., Gray, S. R., Weeks, M., Palmer, M., and Vasiljevic, T. (2016). Nanofiltration and nanodiafiltration of acid whey as a function of pH and temperature. *Separation and Purification Technology*, *160*, 18-27. doi: 10.1016/j.seppur.2015.12.046

6- Cipolat-Gotet, C., Cecchinato, A., De Marchi, M., and Bittante, G. (2013). Factors affecting variation of different measures of cheese yield and milk nutrient recovery from an individual model cheese-manufacturing process. *Journal of Dairy Science*, *96*(12), 7952-7965. doi: 10.3168/jds.2012-6516

7- Durak-Dados, A., Michalski, M., and Osek, J. (2020). Histamine and other biogenic amines in food. *Journal of Veterinary Research*, *64*(2), 281. doi: 10.2478/jvetres-2020-0029

8- Guimarães, P. M., Teixeira, J. A., and Domingues, L. (2010). Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. *Biotechnology advances*, *28*(3), 375-384. DOI: https://doi.org/10.1016/j.biotechadv.2010.02.002

9- Holck, A., Axelsson, L., McLeod, A., Rode, T. M., and Heir, E. (2017). Health and safety considerations of fermented sausages. *Journal of Food Quality*, *2017*(1), 9753894. https://doi.org/10.1155/2017/9753894

10- Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat science*, *78*(1-2), 68-76. https://doi.org/10.1016/j.meatsci.2007.05.030

11- Karageorgou, A., Paveli, A., Goliomytis, M., Theodorou, G., Politis, I., and Simitzis, P. (2023). The effects of yoghurt acid whey marination on quality parameters of pork and chicken meat. *Foods*, *12*(12), 2360. https://doi.org/10.3390/foods12122360

12- Karwowska, M., Kononiuk, A., and Stasiak, D. M. (2022). Effect of Acid Whey in Combination with Sodium Ascorbate on Selected Parameters Related to Proteolysis in Uncured Dry-Fermented Sausages. *Applied Sciences*, *12* (16), 8316. https://doi.org/10.3390/app12168316

13- Karwowska, M., Stadnik, J., Stasiak, D. M., Wójciak, K., and Lorenzo, J. M. (2021). Strategies to improve the nutritional value of meat products: Incorporation of bioactive compounds, reduction or elimination of harmful components and alternative technologies. *International Journal of Food Science and Technology*, *56*(12), 6142-6156. doi: 10.1111/ijfs.15060

14- Kononiuk, A. D., and Karwowska, M. (2019). Influence of freeze-dried acid whey addition on biogenic amines formation in a beef and deer dry fermented sausages without added nitrite. *Asian-Australasian Journal of Animal Sciences*, *33*(2), 332. doi: 10.5713/ajas.19.0011

15- Landete, J. M., Pardo, I., & Ferrer, S. (2008). Regulation of hdc expression and HDC activity by enological factors in lactic acid bacteria. *Journal of applied microbiology*, *105*(5), 1544-1551. doi: 10.1111/j.1365-2672.2008.03865

16- Latorre-Moratalla, M. L., Bover-Cid, S., Veciana-Nogués, M. T., and Vidal-Carou, M. C. (2012). Control of biogenic amines in fermented sausages: role of starter cultures. *Frontiers in microbiology*, *3*, 169. doi: 10.3389/fmicb.2012.00169

17- Latorre-Moratalla, M. L., Comas-Basté, O., Bover-Cid, S., and Vidal-Carou, M. C. (2017). Tyramine and histamine risk assessment related to consumption of dry fermented sausages by the Spanish population. *Food and Chemical Toxicology*, *99*, 78-85. doi: 10.1016/j.fct.2016.11.011

18- Li, J., He, X., Chen, L., Li, J., Yu, X., Li, S., Borjigin, G., Dong, T., and Yun, X. (2025). Effects of whey on quality and differential metabolites of fermented camel jerky. *Food Research International*, *214*, 116678. doi: https://doi.org/10.1016/j.foodres.2025.116678

19- Ma, L., Hu, L., Feng, X., and Wang, S. (2018). Nitrate and nitrite in health and disease. *Aging and disease*, *9*(5), 938. doi: 10.14336/AD.2017.1207

20- Roshanghias, S., and Madadlou, A. (2018). Functional and gel properties of whey protein nanofibrils as influenced by partial substitution with cellulose nanocrystal and alginate. *International Dairy Journal*, *81*, 53-61. DOI: https://doi.org/10.1016/j.idairyj.2018.02.004

21- Schirone, M., Esposito, L., D'Onofrio, F., Visciano, P., Martuscelli, M., Mastrocola, D., and Paparella, A. (2022). Biogenic amines in meat and meat products: A review of the science and future perspectives. *Foods*, *11*(6), 788. DOI: https://doi.org/10.3390/foods11060788

22- Siekmann, L., Plötz, M., and Krischek, C. (2021). Alternative curing methods. *Current Clinical Microbiology Reports*, *8*(2), 40-48. doi: 10.1007/s40588-021-00164-w
23- Tahmouzi, S., Alizadeh Salmani, B., Eskandari, S., and Arab, M. (2025). Effects of plant substitutes for nitrite on the technological characteristics of fermented sausages: A comprehensive review. *Food Science & Nutrition*, *13*(4), e70186. DOI: https://doi.org/10.1002/fsn3.70186
24- Vasconcelos, H., de Almeida, J. M., Matias, A., Saraiva, C., Jorge, P. A., and Coelho, L. C. (2021). Detection of biogenic amines in several foods with different sample treatments: An overview. *Trends in Food Science & Technology*, *113*, 86-96. DOI: 10.1016/j.tifs.2021.04.043
25- Weremfo, A., Eduafo, M. K., Gyimah, H. A., and Abassah-Oppong, S. (2020). Monitoring the levels of biogenic amines in canned fish products marketed in Ghana. *Journal of Food Quality*, *2020*(1), 2684235. DOI: 10.1155/2020/2684235
26- Wherry, B., Barbano, D. M., and Drake, M. A. (2019). Use of acid whey protein concentrate as an ingredient in nonfat cup - style yogurt. *Journal of Dairy Science*, *102* (10), 8768-8784. DOI: https://doi.org/10.3168/jds.2019-16247
27- Zhang, Y., Zhang, Y., Jia, J., Peng, H., Qian, Q., Pan, Z., and Liu, D. (2023). Nitrite and nitrate in meat processing: Functions and alternatives. *Current Research in Food Science*, *6*, 100470. DOI: https://doi.org/10.1016/j.crfs.2023.100470
28- Zotta, T., Solieri, L., Iacumin, L., Picozzi, C., and Gullo, M. (2020). Valorization of cheese whey using microbial fermentations. *Applied microbiology and biotechnology*, *104*(7), 2749-2764. DOI: https://doi.org/10.1007/s00253-020-10408-2

Table 1. *Formulation of fermented sausage (The liquid whey experiment)*

Sausage Ingredients	Percentage
Minced Meat	80%
Fat	15%
Salt	2%
Spices	1%
Starch	5%
T1	Sodium Nitrite 0.005%
T2	Sodium Nitrite 0.005% +Liquid Whey 5ml
T3	Sodium Nitrite 0.002% +Liquid Whey 5ml
T4	Liquid Whey 5ml
T5	Liquid Whey 5ml + Ascorbic acid 0.05%

Table 2. *Formulation of fermented sausage (The dry whey experiment)*

Sausage Ingredients	Percentage
Minced Meat	80%
Fat	15%
Salt	2%
Spices	1%
Starch	5%
T1	Sodium Nitrite 0.005%
T2	Sodium Nitrite 0.005% +Dry Whey 2%
T3	Sodium Nitrite 0.005% +Dry Whey 5%
T4	Sodium Nitrite 0.002% +Dry Whey 2%
T5	Dry Whey 5%
T6	Dry Whey 5% +Ascorbic acid 0.05%.

***Table 3**: Peaks identified in the HPLC chromatographic profile of Standard reference compounds (Biogenic amines)*

Peak	R .time (min.)	Area%	Height%	Compound Name
6	4.760	0.5	0.7	Methylamine
13	8.828	1.3	1.9	Phenylethylamine
16	9.696	2.1	2.9	Putrescine
18	10.424	12.2	13.4	Cadaverine
19	11.304	6.4	6.9	Histamine
25	19.220	3.3	1.9	Tyramine
26	20.024	16.6	16.3	Trimethylamine
27	22.436	0.1	0.1	Spermidine

***Table 4.** Biogenic amine content in sausage treated with liquid whey and ascorbic acid.*

Treatments	The Amount of Biogenic Amine (ppm) (Mean ± SD)							
	Methylamine	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Trimethylamine	Spermidine
T1	4.85±0.005 ^{a}	4.62±0.011^{b}	4.98±0.005^{b}	5.22±0.02^{a}	5.04±0.03^{d}	5.01±0.005^{a}	5.03±0.006^{b}	5.01±0.006^{a}
T2	4.85±0.01 ^{a}	4.88±0.01 ^{a}	4.98±0.01^{b}	4.90±0.57^{a}	5.06±0.006^{d}	ND	5.02±0.021^{b}	5.01±0.006^{a}
T3	4.73±0.02 ^{b}	ND	5.05±0.03^{a}	5.11±0.005^{a}	5.17±0.01^{b}	4.96±0.012^{b}	5.11±0.006^{a}	ND
T4	ND*	ND	4.70±0.005^{c}	5.13±0.01^{a}	5.13±0.006^{c}	ND	5.02±0.021^{b}	ND
T5	4.72±0.026 ^{b}	ND	ND	5.00±0.005^{a}	5.21±0.005^{a}	5.01±0.005^{a}	5.12±0.011^{a}	ND

T1- Sodium Nitrite 0.005% ; T2- Sodium Nitrite 0.005%+Liquid Whey 5ml ; T3- Sodium Nitrite 0.002% +Liquid Whey 5ml , T4- Liquid Whey 5ml ; T5- Liquid Whey 5ml+ Ascorbic acid 0.05 %. Different letters within the same column indicate significant differences at $p \leq 0.01$. *ND(Not Detected)

***Table 5**. Biogenic amine content in sausage treated with dry whey and ascorbic acid.*

Treatments	The Amount of Biogenic Amine (ppm) (Mean ± SD)							
	Methylamine	Phenylethylamine	Putrescine	Cadaverine	Histamine	Tyramine	Trimethylamine	Spermidine
T1	4.85±0.005^{b}	4.62±0.011^{d}	4.98±0.01^{b}	5.22±0.01^{b}	5.04±0.01^{cd}	5.01±0.005^{a}	5.03±0.02^{b}	5.01±0.005^{a}
T2	4.50±0.005^{c}	4.91±0.01^{b}	4.98±0.01^{b}	5.85±0.02^{a}	5.12±0.03^{c}	5.00±0.005^{a}	5.01±0.005^{b}	ND
T3	4.51±0.01^{c}	4.98±0.01^{a}	4.92±0.01^{c}	4.96±0.012^{c}	5.08±0.01^{c}	ND	ND	ND
T4	4.87±0.01^{a}	4.67±0.005^{c}	5.10±0.01^{a}	ND	5.28±0.01^{a}	5.06±0.005^{a}	5.15±0.01^{a}	4.97±0.01^{b}
T5	ND	4.98±0.01^{a}	ND	ND	5.21±0.01^{b}	ND	5.17±0.01^{a}	ND
T6	4.88±0.01^{a}	ND	ND	ND	ND	ND	ND	ND

T1- Sodium Nitrite 0.005% ; T2- Sodium Nitrite 0.005%+Dry Whey 2% ; T3- Sodium Nitrite 0.005% + Dry Whey 5%; T4- Sodium Nitrite 0.002%+Dry Whey 2% ; T5-Dry Whey 5%; T6- Dry Whey 5% +Ascorbic acid 0.05%. Different letters within the same column indicate significant differences at $p \leq 0.01$.*ND(Not Detected)

Table 6. *The amount of residual nitrite in sausage treated with liquid whey and ascorbic acid*

Treatments	The amount of Residual nitrite (ppm) (Mean ± SD)
T1	2.34±0.006 [a]
T2	1.31±0.006 [b]
T3	1.13±0.015 [c]
T4	0.81±0.006 [d]
T5	0.71±0.005 [e]

T1- Sodium Nitrite 0.005% ; T2- Sodium Nitrite 0.005%+Liquid Whey 5 ml ; T3- Sodium Nitrite 0.002%+Liquid Whey 5 ml , T4- Liquid Whey 5 ml ; T5- Liquid Whey 5 ml+ Ascorbic acid 0.05%. Different letters within the same column indicate significant differences at $p \leq 0.01$.

Table 7. *The amount of residual nitrite in sausage treated with Dry whey and ascorbic acid*

Treatments	The amount of Residual nitrite (ppm) (Mean ± SD)
T1	2.34±0.006 [a]
T2	1.41±0.006 [c]
T3	1.71±0.015 [b]
T4	1.31±0.006 [c]
T5	0.73±0.01 [d]
T6	0.71±0.005 [d]

T1- Sodium Nitrite 0.005% ; T2- Sodium Nitrite 0.005%+Dry Whey 2% ; T3- Sodium Nitrite 0.005%+Dry Whey 5%; T4- Sodium Nitrite 0.002%+Dry Whey 2% ; T5-Dry Whey 5%; T6- Dry Whey 5% +Ascorbic acid 0.05%. Different letters within the same column indicate significant differences at $p \leq 0.01$.

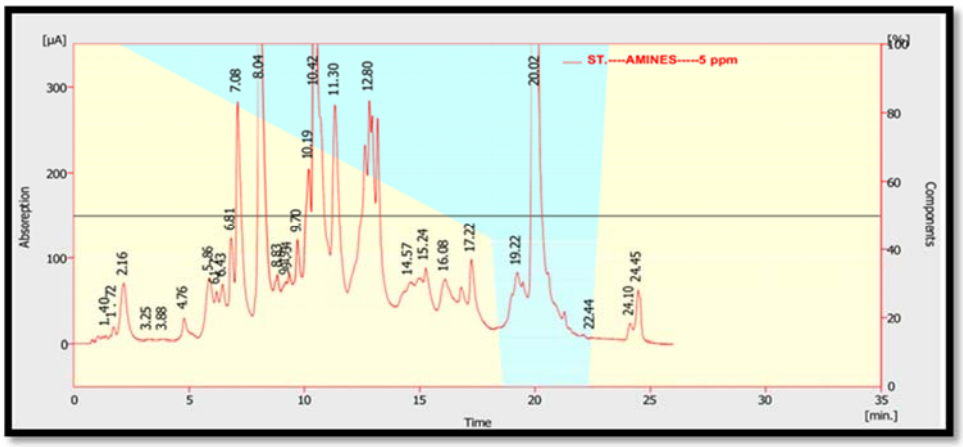

Figure 1: *HPLC Chromatogram of Standard reference compounds*

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