

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 التخزين 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

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.*

المخلص:

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 تقصي تأثير إضافة مصّل اللين السائل الحمضي ومصّل اللين الجاف، مع حمض الأسكوربيك، في مستويات الأمينات الحيوية وقياس النترت المتبقي في النقانق الطازجة والمخزنة تحت التبريد. **طرائق العمل:** اشتملت الدراسة على تجربتين: التجربة الأولى تضمّنّت خمس معاملات: T1- نترت الصوديوم 0.005%؛ T2- نترت الصوديوم 0.005% + مصّل اللين السائل 5 مل؛ T3- نترت الصوديوم 0.002% + مصّل اللين السائل 5 مل؛ T4- مصّل اللين السائل 5 مل؛ T5- مصّل اللين السائل 5 مل + حمض الأسكوربيك 0.05%. أما التجربة الثانية فقد شملت ست معاملات: T1- نترت الصوديوم 0.005%؛ T2- نترت الصوديوم 0.005% + مصّل اللين الجاف 2%؛ T3- نترت الصوديوم 0.005% + مصّل اللين الجاف 5%؛ T4- نترت الصوديوم 0.002% + مصّل اللين الجاف 2%؛ T5- مصّل اللين الجاف 5%؛ T6- مصّل اللين الجاف 5% + حمض الأسكوربيك 0.05%. وبعد عملية التصنيع وفترة خزن بلغت 21 يومًا، جرى تقييم مستويات الأمينات الحيوية والنترت المتبقي. **النتائج:** أدت إضافة كلّ من مصّل اللين السائل الحمضي ومصّل اللين الجاف، إلى جانب حمض الأسكوربيك، إلى خفض مستويات الأمينات الحيوية في النقانق. كما لوحظت فروق معنوية بين المعاملة التي احتوت على نترت الصوديوم فقط وتلك التي احتوت على مصّل اللين السائل الحمضي أو مصّل اللين الجاف، فضلًا عن المعاملات التي جمعت بين حمض الأسكوربيك وأحد هذين النوعين من مصّل اللين. كما أظهرت النتائج انخفاضًا في مستويات النترت المتبقي في النقانق المعالجة بمصّل اللين السائل الحمضي ومصّل اللين الجاف مع حمض الأسكوربيك، مقارنةً بالمعاملة التي تضمّنّت نترت الصوديوم فقط. **المناقشة:** أسهمت هذه الإضافات في إنتاج منتجات لحوم مصنّعة تحتوي على أقل مستويات من الأمينات الحيوية وأدنى قيم للنترت المتبقي. وبناءً على ذلك، يُوصى باستخدام كلّ من مصّل اللين السائل والجاف، إلى جانب حمض الأسكوربيك، في منتجات اللحوم المصنّعة بوصفها بديلًا عن النيتريتات. **الاستنتاجات:** يُعدّ استخدام كلّ من مصّل اللين السائل والجاف في إنتاج النقانق استراتيجية مبتكرة لتطوير منتجات لحوم خالية من النيتريت، بما يتماشى مع اتجاه "الملصق النظيف".

الكلمات المفتاحية: مصّل اللين، حمض الاسكوربيك، الامينات الحيوية، النترت 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; Chandraqaola *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 4.92 ppm and 5.01 ppm, 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 *et 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

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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. 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

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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.

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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 $p \leq 0.01$.

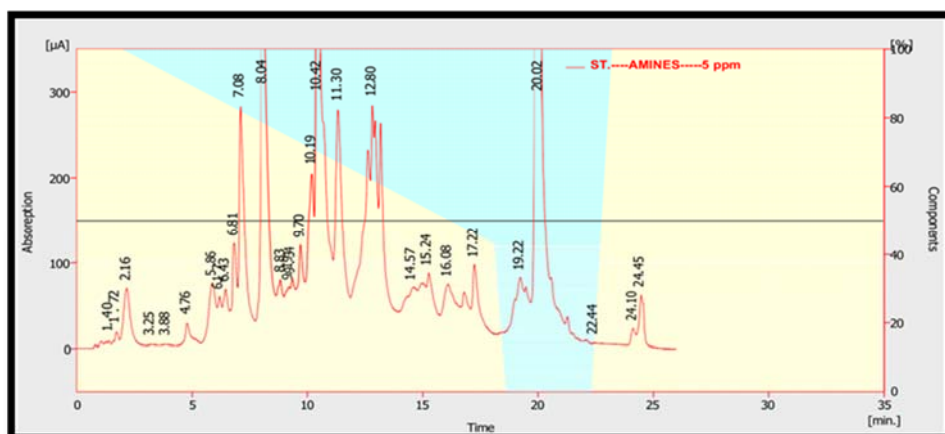


Figure 1: HPLC Chromatogram of Standard reference compounds