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# Starch, nitrite, and nitrate analysis in inspected and uninspected fresh pork sausages

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**Abstract**

Fresh pork sausages are among the most consumed meat products in Brazil. However, in the informal market, they are more susceptible to fraud by adding prohibited ingredients, such as starch, or in amounts exceeding safe health limits. In this context, the present study quantified the concentrations of nitrite and nitrate and also investigated the presence of starch in samples of fresh pork sausages sold in the mesoregion of the municipality of Bauru, São Paulo, Brazil. A total of 40 samples were analyzed, including 30 from three different brands inspected by regulatory agencies and 10 uninspected. All food products analyzed were within the limits established by Brazilian legislation for starch (absence), nitrite, and nitrate (maximum of 150 and 300 mg/kg, respectively). The importance of consuming inspected food is emphasized, given the health risks posed by the consumption of products that do not comply with the hygienic-sanitary and production standards established by law.

**Keywords:** public health; food safety; curing salts; fraud.

**Practical Application:** Monitoring additives in sausages supports food safety and guides public health policies.

## 1 INTRODUCTION

Over the years, significant changes in people's eating habits have been observed. The high consumption of processed foods stands out as an important risk factor for the development of non-communicable chronic diseases, such as neoplasms (Barreto et al., 2005). With the increasing demand for processed products, food technology has increasingly invested in methods to preserve these resources, extend their shelf life, and meet market expectations. In this context, the use of food additives has become increasingly common (Moutinho et al., 2007).

According to the World Health Organization (2019), food additives are substances, with or without nutritional value, that are not used as basic ingredients but are intentionally added to foods to preserve or promote a desired characteristic (WHO, 2019). Despite their benefits, various studies have identified food additives as potential causes of acute and chronic illnesses. In humans, allergic reactions, carcinogenic effects, and neurological alterations have been reported, with children being the most vulnerable group (Wilson & Bahna, 2005). Therefore, strict control over the amount of these substances added to food is essential (Evangelista, 2000; Pollock, 1991).

Sodium and potassium nitrate and nitrite are additives widely used in meat products. They are capable of inhibiting the growth of spoilage microorganisms, fixing the reddish color that enhances commercial appeal, participating in the curing process (salting and the development of specific sensory characteristics), delaying fat oxidation, and inhibiting pathogenic bacteria such as *Clostridium botulinum* (Flores & Toldrá, 2021). However, when consumed in high amounts, they may cause adverse effects, such as the conversion of hemoglobin into methemoglobin, which compromises the function of red blood cells, vasodilatory effects, and the formation of nitrosamines—compounds that are potentially carcinogenic over the long term (Cammack et al., 1999; Honikel, 2008).

Approximately 70% of the pork consumed in Brazil is in the form of processed foods (Mürmann et al., 2009). Among these, sausages are particularly prominent. Evidence suggests that pork sausages present a higher risk of contamination and microbial proliferation than fresh meat cuts. This is mainly attributed to the handling steps involved in preparing and storing these processed products, which contribute to the high contamination levels found in this type of food (Mürmann et al., 2009).

The use of curing salts is a strategy to prevent foodborne pathogens. Sullivan et al. (2012) observed that the growth of *Clostridium perfringens* and *Listeria monocytogenes* was more pronounced in meat products not treated with nitrite compared to traditionally cured ones. Therefore, the absence of this salt in processed sausages may also pose public health risks.

Fresh pork sausages are often produced and sold informally, without proper inspection or sanitary control by government agencies. For this reason, they are susceptible to fraud, such as the intentional addition of starch, a polysaccharide that affects texture and increases volume (Pedroso & Demiate, 2008).

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The study aimed to evaluate the presence of starch and the concentrations of nitrite and nitrate ions in fresh pork sausages, both inspected and uninspected by federal, state, or municipal regulatory agencies, sold in the Bauru Mesoregion, São Paulo, Brazil.

### *1.1 Relevance of the work*

This study highlights the importance of regulatory inspection in ensuring the safety and legal compliance of fresh pork sausages, reinforcing public health protection through monitoring of additives and prohibited substances.

## 2 MATERIALS AND METHODS

### *2.1 Samples*

The analyzed samples consisted of fresh pork sausages, both inspected and uninspected by regulatory agencies, sold in bulk at supermarkets and butcher shops in the mesoregion of the municipality of Bauru, São Paulo, Brazil. Three groups (brands) of inspected fresh pork sausages and one group of uninspected fresh sausages were analyzed. The codes for the sausage groups were as follows: (a) inspected sausage from brand A (ISA; $n$ = 10); (b) inspected sausage from brand B (ISB; $n$ = 10); (c) inspected sausage from brand C (ISC; $n$ = 10); and (d) uninspected sausage (NIS; $n$ = 10). In total, 40 samples were analyzed.

The samples were transported in refrigerated thermal boxes, maintained between 0 and 4°C, to the Food Physicochemistry Laboratory of the Public Food Guidance Service (SOAP), Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP—Botucatu Campus). All assays described in this study were validated by the laboratory. The physicochemical analyses were performed in triplicate.

### *2.2 Starch analysis*

A total of 5 g of the homogenized sample was weighed into a 100 mL glass beaker. Twenty milliliters of purified water was added and homogenized using a glass rod. The beaker's contents were heated over a Bunsen burner. Once boiling started, the heating was timed for 5 min. After cooling, 10 drops of iodine tincture solution were added. The resulting color was observed. In the absence of starch, the color remained in shades of brown. In the presence of starch, blue tones were expected to appear. According to the methodology, if the qualitative test was positive, a quantitative analysis would be carried out using the Lane-Eynon method, as described by the Instituto Adolfo Lutz (IAL, 2008).

### *2.3 pH analysis*

A total of 10 g of the homogenized sample was weighed into a 100 mL glass beaker. Then, 20 mL of purified water was added, and the mixture was homogenized using a glass rod. The pH of the sample was determined using a benchtop pH meter, brand Kasvi®, model series K39-2014B (Paraná, Brazil). The equipment was calibrated before each use, following the standard operating procedure recommended by the manufacturer and the laboratory. After calibration, the electrode was inserted into the samples, and the pH reading was taken once the values stabilized (± 5 min).

### *2.4 Nitrite analysis*

A total of 10 g of the homogenized sample was weighed into a 250 mL Erlenmeyer flask with a ground-glass stopper. Then, 5 mL of 5% sodium tetraborate decahydrate solution and 50 mL of purified water were added. The flask was placed in a water bath at 80°C for 20 min with frequent stirring. Using a glass rod and a funnel, the contents were quantitatively transferred to a 200 mL volumetric flask, washing the Erlenmeyer and funnel repeatedly with purified water. Next, 5 mL of 15% potassium ferrocyanide solution and 5 mL of 30% zinc sulfate solution were added, with stirring after each addition. The volumetric flask was filled to 200 mL with purified water. The mixture was left to stand for 15 min, with agitation every 3 min. The contents were then filtered through qualitative filter paper into a clean, dry flask.

After filtration, a 10 mL aliquot of the filtrate was transferred to a 50 mL graduated cylinder with a ground-glass stopper. Then, 5 mL of 0.5% sulfanilamide reagent and 3 mL of 0.5% NED (alpha-naphthylethylenediamine dihydrochloride) reagent were added. The cylinder was filled to 50 mL with purified water. The solution was homogenized and allowed to stand for 15 min.

The absorbance reading was performed using a ultraviolet-visible (UV-Vis) spectrophotometer (Femto® brand, model 600 Plus, São Paulo, Brazil) at 540 nm, using a 10-mm optical path methacrylate cuvette. The nitrite concentration was calculated based on the standard curve established by the laboratory during method validation, using Equation 1 (Martins & Graner, 2005a, 2005b, 2005c):

$$\text{Nitrite ion (mg/kg)} = [(\text{Absorbance} - 0.0134) \times 1000]/(\text{mass} \times 0.6993) \quad (1)$$

### *2.4 Nitrate analysis*

#### *2.4.1 Preparation of metallic cadmium*

A total of 15 g of metallic cadmium (Merck; Ref. 1.02001; 0.3–1.6 mm) was weighed into a 250-mL glass beaker. Approximately 25 mL of 2 M hydrochloric acid solution was added, enough to cover the cadmium. The mixture was homogenized using a glass rod and allowed to stand for 2 min. The supernatant was discarded as chemical waste. The cadmium was washed approximately six times with purified water. The pH of the washing water was checked using pH indicator strips (Merck; Ref. 1.09535; pH 0–14) and confirmed to be around pH 7. After discarding the final wash water, approximately 25 mL of 0.1 M hydrochloric acid solution was added to cover the cadmium. The mixture was homogenized and left to stand for 15 min. The supernatant was then discarded, and the cadmium was washed about six times

with purified water again, checking the pH each time. After discarding the last portion of wash water, approximately 25 mL of cadmium buffer solution (pH 9.3–9.7) was added to cover the cadmium and left for 15 min. After this period, the cadmium was ready for use in the analytical assay. All water used in the washing process was treated as chemical waste.

*2.4.2 Spectrophotometric determination of nitrate ion*

The cadmium was weighed into a 100-mL Erlenmeyer flask with a ground-glass stopper. Then, 20 mL of the filtrate obtained in Section 2.4 was transferred into the flask. Subsequently, 5 mL of buffer solution (pH 9.6–9.7) and 2 mL of 5% ethylenediaminetetraacetic acid (EDTA) solution were added. The flask was homogenized using a shaker (Fisatom® brand, model 786; Brazil) for 20 min. The supernatant was quantitatively transferred into a 100-mL graduated cylinder with a ground-glass stopper, and the volume was completed with purified water. The solution was homogenized, and 10 mL was transferred to a 50-mL graduated cylinder with a ground-glass stopper. Then, 5 mL of 0.5% sulfanilamide reagent was added and allowed to stand for 5 min. After that, 3 mL of 0.5% NED reagent was added. The volume was completed to 50 mL with purified water, and the solution was homogenized. It was then left to stand for 15 min.

Approximately 2.5 mL of the solution was transferred to a 10 mm optical path methacrylate cuvette. Absorbance was measured using a UV-Vis spectrophotometer (Femto® brand, model 600 Plus, São Paulo, Brazil) at 540 nm. The nitrate value was calculated based on the standard curve established by the laboratory during method validation. The absorbance value was applied to the following formula (Equation 2):

$$\text{Total nitrite ion (mg/kg)} = [(\text{Absorbance} - 0.0134) \times 5000]/(\text{mass} \times 0.6993) \quad (2)$$

To calculate the nitrate ion concentration, the following equation was used (Equation 3) (Martins & Graner, 2005a, 2005b, 2005c):

$$\text{Nitrate ion (mg/kg)} = \text{Total nitrite ion (mg/kg)} - \text{Nitrite ion (mg/kg)} \quad (3)$$

*2.4.3 Reuse of cadmium*

The cadmium used in the analyses was transferred to a glass beaker and washed with purified water. The wash water was discarded as chemical waste. The cadmium was dried in an oven at 37°C ± 2°C. After drying, it was stored in an appropriate container and reused for subsequent analyses.

### *2.5 Statistical analysis*

The values obtained from the assays were statistically analyzed using analysis of variance (ANOVA) based on a completely randomized design, followed by Tukey's test for mean comparisons, considering a significance level of 5% (Montgomery, 2020). Absolute frequency (AF), relative frequency (RF), and relative frequency in percentage (RF%) were used to analyze the starch detection results.

## 3 RESULTS

### *3.1 Starch analysis*

None of the analyzed fresh pork sausage samples (40/40) tested positive for the presence of starch (Table 1).

### *3.2 pH analysis*

There was no statistically significant difference in pH values among the different groups of fresh pork sausage analyzed. The results of the ANOVA and the mean values obtained are presented in Tables 2 and 3, respectively. The mean pH values for the ISA, ISB, ISC, and NIS sample groups were 5.69 ± 0.75, 5.85 ± 0.44, 5.84 ± 0.14, and 5.39 ± 0.27, respectively (Table 3).

**Table 1**. Absolute frequency (AF), relative frequency (RF), and relative frequency in percentage (%) of the starch presence (present or absent) in the samples from the ISA, ISB, ISC, and NIS groups.

| Group (n) | Result | Absolute frequency (AF) | Relative frequency (RF) | Relative frequency (%) |
|---|---|---|---|---|
| ISA (10) | Present | 0 | 0.00 | 0 |
| | Absent | 10 | 0.25 | 25 |
| ISB (10) | Present | 0 | 0.00 | 0 |
| | Absent | 10 | 0.25 | 25 |
| ISC (10) | Present | 0 | 0.00 | 0 |
| | Absent | 10 | 0.25 | 25 |
| NIS (10) | Present | 0 | 0.00 | 0 |
| | Absent | 10 | 0.25 | 25 |
| **Total** | | **40** | **1.00** | **100** |

**Table 2**. Analysis of variance (ANOVA) for the determination of pH in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausage samples.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | P-value |
|---|---|---|---|---|
| Treatments | 3 | 1.41111 | 0.47037 | 2.26 ($p > 0.05$) |
| Residual | 36 | 7.6492 | 0.21247 | |

Coefficient of variation: 8.1%.

**Table 3**. Mean ± standard deviation of pH values in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausage samples. Statistical analysis complemented with Tukey's test at a 5% significance level.

| Group | Mean ± SD |
|---|---|
| ISA | 5.69 ± 0.75 a[(1)] |
| ISB | 5.85 ± 0.44 a |
| ISC | 5.84 ± 0.14 a |
| NIS | 5.39 ± 0.27 a |

SD: standard deviation; Coefficient of variation: 8.10%; Honestly significant difference (5%): 0.55; [(1)] Tukey's test: no significant differences ($p > 0.05$).

### *3.3 Nitrite analysis*

The results of the ANOVA and the mean nitrite values found in the different groups of fresh pork sausages are presented in Tables 4 and 5, respectively. No statistically significant differences were observed among the inspected sausage brands; however, the non-inspected group showed significant variation when compared to the inspected ones (Table 5). The mean nitrite values for the ISA, ISB, ISC, and NIS sample groups were 1.97731 ± 3.08100, 4.68071 ± 4.62677, 2.84070 ± 3.10477, and 34.42000 ± 35.65392 mg/kg, respectively (Table 5).

### *3.4 Nitrate analysis*

The ANOVA and the mean nitrate values found in the fresh pork sausage samples are presented in Tables 6 and 7, respectively. The inspected brands, ISA and ISB, did not show statistically significant differences between each other. However, the ISC (inspected) and NIS (non-inspected) brands showed significant statistical differences between them (Table 7). The mean nitrate values for the ISA, ISB, ISC, and NIS sample groups were 1.43511 ± 4.53822, 0.47190 ± 1.49228, 0.05663 ± 0.17908, and 18.41077 ± 32.27274, respectively (Table 7).

## 4 DISCUSSION

Starch is a carbohydrate widely used in the formulation of certain meat products due to its low cost, water-retention properties, and its function as a thickening and stabilizing agent, influencing the texture of foods to which it is added (Pedroso & Demiate, 2008). Despite these advantages, its use in certain food products is considered fraudulent. In the case of fresh pork sausages, the mandatory ingredients are inspected meat from different animal species and salt, encased in either natural or artificial casings and subjected to appropriate technological processing, with the addition of starch being prohibited (Brasil, 2000).

None of the samples analyzed in this study (100%, 40/40) (Table 1) contained starch in their composition, according to the current Brazilian legislation.

pH is an important physicochemical parameter in meat products, as it reflects both the microbiological and technological quality of the meat. According to Pardi et al. (2007), an acidic pH favors food safety by inhibiting the growth of various pathogens. However, from a technological standpoint, a rapid or excessive drop in pH can be detrimental. This occurs when animals are subjected to intense pre-slaughter stress, resulting in PSE (pale, soft, and exudative) meat, which presents undesirable sensory characteristics in the market. Although there is no defined ideal pH value for sausage products, the Brazilian Regulation for the Industrial and Sanitary Inspection of Products of Animal Origin (Brasil, 1952) establishes a pH range of 6.0–6.4 as acceptable for meat in general, considering meat within this range suitable for consumption.

All samples evaluated (100%, 40/40) fell within the pH range established by Brazilian legislation. The mean pH values for the ISA, ISB, ISC, and NIS sample groups were 5.69 ± 0.75, 5.85 ± 0.44, 5.84 ± 0.14, and 5.39 ± 0.27, respectively (Table 3).

**Table 4**. Analysis of variance (ANOVA) for the determination of nitrite ion in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausages.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | P-value |
|---|---|---|---|---|
| Treatments | 3 | 7,363.84244 | 2,454.61415 | 7.49 ($p < .001$) |
| Residual | 36 | 11,805.6661 | 327.93517 | |

Coefficient of variation: 164.93%.

**Table 5**. Mean ± standard deviation of nitrite ion values (mg/kg) in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausages. Statistical analysis complemented with Tukey's test at a 5% significance level.

| Group | Mean ± SD |
|---|---|
| ISA | 1,97731 ± 3,08100 a[(1)] |
| ISB | 4,68071 ± 4,62677 a |
| ISC | 2,84070 ± 3,10477 a |
| NIS | 34,42000 ± 35,65392 b |

SD: standard deviation; Coefficient of variation: 164.93%; Honestly significant difference (5%): 21.7; [(1)] Tukey's test: $p > .001$.

**Table 6**. Analysis of variance (ANOVA) for the determination of nitrate ion in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausages.

| Source of variation | Degrees of freedom | Sum of squares | Mean square | P-value |
|---|---|---|---|---|
| Treatments | 3 | 2,374.62754 | 791.54251 | 2.97 ($p < .05$) |
| Residual | 36 | 9,579.45476 | 266.09597 | |

Coefficient of variation: 320.25%.

**Table 7**. Mean ± standard deviation of nitrate ion values (mg/kg) in inspected (ISA, ISB, and ISC) and non-inspected (NIS) fresh pork sausages. Statistical analysis complemented with Tukey's test at a 5% significance level.

| Group | Mean ± SD |
|---|---|
| ISA | 1.43511 ± 4.53822 ab[(1)] |
| ISB | 0.47190 ± 1.49228 ab |
| ISC | 0.05663 ± 0.17908 a |
| NIS | 18.41077 ± 32.27274 b |

SD: standard deviation; Coefficient of variation: 320.25%; Honestly significant difference (5%): 18.35; [(1)]Tukey's test: $0.01 > p < .05$.

Curing salts, composed of nitrite and nitrate, are used in meat products to impart desirable sensory characteristics, such as color, aroma, and flavor, in addition to acting as preservatives by inhibiting the growth of spoilage and pathogenic microorganisms such as *C. botulinum* (Cherobin et al., 2023; Flores & Toldrá, 2021). However, at high concentrations, these additives can be harmful to consumer health (Cammack et al., 1999; Honikel, 2008). Brazilian legislation sets acceptable and safe limits for these components in cured meat products: 150 mg/kg for nitrite and 300 mg/kg for nitrate (Brasil, 1998, 1999).

Matera et al. (2014), when comparing the physicochemical parameters of artisanal and inspected pork sausages, observed differences between these two categories of products. Sausages inspected by government agencies showed greater uniformity in nitrite content compared to uninspected ones.

The authors also emphasized the need for further studies comparing these two types of sausages in different regions of Brazil, aiming to improve the quality of food products offered to the population.

All the samples evaluated (100%, 40/40) were within the limits established by current Brazilian legislation. The mean nitrite values for the ISA, ISB, ISC, and NIS groups were 1.97731 ± 3.08100, 4.68071 ± 4.62677, 2.84070 ± 3.10477, and 34.42000 ± 35.65392, respectively (Table 5). As for nitrate, the mean values were 1.43511 ± 4.53822, 0.47190 ± 1.49228, 0.05663 ± 0.17908, and 18.41077 ± 32.27274, respectively (Table 7).

## 5 CONCLUSION

All fresh pork sausages analyzed were within the limits established by Brazilian law regarding starch, pH, nitrite, and nitrate parameters. Although the non-inspected products evaluated complied with the physicochemical standards defined by legislation, their commercialization is concerning, as they may pose health risks to consumers due to the lack of strict hygiene and production standards during manufacturing and storage processes.

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