NITRITE AND NITRATE LEVELS IN SAUSAGES

Concentrações de nitrito e nitrato em salsichas

Concentraciones de nitrito y nitrato en salchichas

Original Article

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ABSTRACT

Objective: To determine nitrite and nitrate levels, hydrogen potential (pH), and water activity (aw) in sausages marketed in Southern Brazil, and compare the levels among different brands. **Methods**: Quantitative experimental cross-sectional study conducted with 72 sausage samples collected in two municipalities in the Região do Vale do Taquari, Rio Grande do Sul,,between June and August 2015. Three samples from different batches of each eight distinct sausage brands which were supervised by the Federal Inspection Service were analyzed in triplicate according to their nitrite and nitrate levels, pH and water activity. Data underwent ANOVA and Chi-squared tests with $p<0.05$. **Results**: Samples met the recommended standards for pH and water activity (6.33 ± 0.32 and 0.91 ± 0.01, respectively), while 40.3% (29) and 50.0% (36) of the samples presented nitrite and nitrate levels, respectively, significantly above what is recommended by the legislation ($p<0.05$). The mean nitrite levels of the remaining samples were significantly lower ($p=0.001$), and mean nitrate levels were significantly higher ($p=0.009$) than the value established by legislation. **Conclusion**: The samples met the recommended levels of pH and water activity. Of eight brands assessed, three presented satisfactory levels of nitrate and nitrite. The mean levels of nitrite and water activity were significantly lower and the mean levels of nitrate and pH were significantly higher than the recommendations. Additionally, mean nitrate and nitrite levels of five brands were significantly higher than what is recommended by the legislation.

Descriptors: Sodium Nitrite; Food Preservatives; Legislation, Food.

RESUMO

Objetivo: *Determinar as concentrações de nitrito e nitrato, potencial hidrogeniônico (pH) e atividade de água (Aa) em salsichas comercializadas em uma região do sul do Brasil, e comparar essas concentrações entre as diferentes marcas.* **Métodos:** *Estudo experimental, transversal, quantitativo, realizado com 72 amostras de salsicha coletadas em dois municípios da Região do Vale do Taquari/RS, entre junho e agosto de 2015. Avaliaram-se três amostras de lotes diferentes, por meio das variáveis de nitrito, nitrato, pH e atividade de água, em triplicata, de cada uma das oito marcas de salsichas fiscalizadas pelo Serviço de Inspeção Federal. Dados submetidos aos testes ANOVA e Qui-quadrado, com p<0,05.* **Resultados:** *A totalidade das amostras apresentou-se dentro dos padrões para pH e Aa (6,33 ± 0,32 e 0,91 ± 0,01, respectivamente), enquanto 40,3% (29) e 50,0% (36) apresentaram níveis de nitrito e nitrato, respectivamente, significativamente acima do estabelecido pela legislação (p<0,05). A média de nitrito das amostras restantes apresentou-se significativamente inferior (p=0,001), e a média de nitrato, significativamente superior (p=0,009) ao valor estabelecido pela legislação.* **Conclusão:** *A totalidade das amostras estava adequada em relação aos níveis de pH e Aa. Do total das oito marcas avaliadas, três se encontravam em condições satisfatórias referente à adição do nitrato e nitrito. A média de nitrito e Aa foi significativamente inferior, e as médias do nitrato e pH, significativamente superior, enquanto os níveis de nitrato e nitrito apresentaram médias superiores aos valores estabelecidos legislação, em cinco marcas analisadas.*

Descritores: *Nitrito de Sódio; Conservantes de Alimentos; Legislação sobre Alimentos.*

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RESUMEN

Objetivo: *Determinar las concentraciones de nitrito y nitrato, del potencial de hidrógeno (pH) y de la actividad del agua (Aa) de salchichas del comercio de una región del sur de Brasil y compararlas entre las distintas marcas comerciales.* **Métodos:** *Estudio experimental, transversal y cuantitativo realizado con 72 muestras de salchichas recogidas en dos municipios de la Región del Vale del Taquari/RS entre junio y agosto de 2015. Se evaluaron tres veces tres muestras de lotes distintos a través de las variables de nitrito, nitrato, pH y actividad del agua de cada una de las ocho marcas de salchichas fiscalizadas por el Servicio de Inspección Federal. Los datos fueron sometidos a las pruebas ANOVA y Chi-cuadrado para p<0,05.* **Resultados:** *La totalidad de las muestras se presentó dentro de los patrones para el pH y la Aa (6,33 ± 0,32 e 0,91 ± 0,01, respectivamente) mientras el 40,3% (29) y el 50,0% (36) presentaron niveles de nitrito y nitrato, respectivamente, significativamente por encima de la legislación (p<0,05). La media de nitrito de las muestras restantes se presentó significativamente inferior (p=0,001) y la media de nitrato, significativamente superior (p=0,009) de la legislación.* **Conclusión:** *La totalidad de las muestras estaba adecuada respecto a los niveles de pH y Aa. Del total de las ocho marcas evaluadas, tres se encontraban en condiciones satisfactorias de adicción del nitrato y nitrito. La media de nitrito y Aa fue significativamente inferior y las medias de nitrato y pH significativamente superior mientras los niveles de nitrato y nitrito presentaron medias superiores de la legislación en cinco marcas analizadas.*

Descriptores: *Nitrito de Sodio; Conservantes de Alimentos; Legislación sobre Alimentos.*

INTRODUCTION

Consumers' lifestyle has changed considerably in recent years, with a tendency to prefer foods that are easy and quick to prepare such as chicken and beef hot dogs, which have gained notoriety for its great acceptability[1]. Among other factors, the affordable price of some brands, the simplicity of preparation and the protein content of this product help to reduce malnutrition, especially among low-income consumers[2]. The sausage is the lead product of encased meats with 27% of the total production due to increased consumption of hot dogs, which is the most sold type of food ready for consumption[3].

The sausage, according to Normative Instruction No. 4 from 31 March 2000[4], is a processed meat product obtained from a meat emulsion of one or more species of slaughter animals added to other ingredients and encased in natural or artificial casings or through extrusion process subjected to an appropriate thermal process. The sausages may also be subjected to dyeing and smoking processes and the use of fillings and sauces[4]. Additives such as nitrates and nitrites are added in the manufacturing process with the purpose of improving the sensory characteristics of the product, preserve and increase the life-cycle, inhibit the growth of pathogenic micro-organisms such as *Clostridium botulinum*, and delay lipid oxidation[5,6].

However, the excessive consumption of these additives has worried the scientific community given the harmful effects on human health[7] – the formation of carcinogenic chemical compounds such as nitrosamines and nitrosamides[8], some of which present, in addition to the carcinogenic activity, mutagenic, teratogenic and embryopathy effects[9]. Another harmful effect refers to the methemoglobinemia or Blue Baby Syndrome – when nitrate is present in the organism, especially in children, it acts on hemoglobin causing iron (II) iron (III) ions oxidation and preventing the normal function of hemoglobin, which is responsible for transporting oxygen; such disease can lead to anoxia and death[10-12].

Thus, given the industrial importance and the possible toxicological aspects of these additives, it is necessary to implement the monitoring of their levels through the quantitative determinations of these compounds, avoiding major risks to consumers' health[13,14]. In order to control the levels of these salts, the Brazilian legislation establishes maximum permitted levels of sodium nitrite and nitrate (preservatives) – 150 mg/kg and 300 mg/kg, respectively – for meat and meat products[15,16].

With regard to water activity, which is an indicator that determines the water available in food for chemical and enzymatic reactions and microbial growth[17], there are no values established by the legislation[4]. However, it can be an important indicator of microbial activity given that this parameter is complementary to determine shelf life, with reference values above 0.88 for water activity standard[18]. The hydrogenionic potential (pH) is also associated with the development of microorganisms in any food and is considered an intrinsic factor of the food; however, low-acid foods (pH above 4.5) such as sausages are more vulnerable to microbial growth[17].

The present study is intended to show the importance of periodic analysis of the levels of nitrites and nitrates, as they are an important tool for the control of the quality of foods consumed by the population in order to promote health, as these additives can have toxic effects on individuals exposed to them through food, depending on the amount consumed and the body's susceptibility[14].

The present study aimed to determine nitrite and nitrate levels, hydrogen potential (pH), and water activity (aw) in sausages marketed in Southern Brazil, and compare the levels among different brands.

METHODS

This is a quantitative experimental cross-sectional study conducted in the Região do Vale do Taquari, Rio Grande do Sul, between June and August 2015. The study used 72 samples of chicken sausages collected at the four major shopping facilities in two cities located in this region.

Three batches of eight samples from six different brands were collected. Only one of the brands belonged to the same manufacturer, and the analyses were performed in triplicate. The production of the brands of sausages was supervised by the Federal Inspection Service (*Serviço de Inspeção Federal – SIF*).

The sausages were obtained from intact and sealed packages in the supermarkets and were transported to the analysis laboratory in boxes refrigerated at 4 °C. After arriving at the laboratory, each package was divided into four samples of 30g – each of them was packaged and identified according to the supermarket, batch and date of collection; after that, they were stored in a refrigerator at 4 °C until the time of analysis, which took place in a maximum of two days. The analyses were performed at the Chemistry Laboratory of the Univates University Center (*Centro Universitário Univates*) by a lab technician trained by the researcher in charge of the laboratory.

The analysis of residual levels of nitrites and nitrates, pH and water activity in the samples were performed according to official analytical methods recommended by the Normative Instruction No. 20, of 21 July 1999, of the Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento – MAPA*)[19].

Nitrite and nitrate analysis were performed considering the cutoff values of 150 and 300 mg/kg, respectively; pH analysis considered the value of 4.5 recommended by the regulations of low-acid foods and water activity was controlled to values greater than 0.88[18].

Statistical analysis was performed by creating categories for nitrate, nitrite, pH and water activity variables as for their compliance with regulations when appropriate; the analyses were based on studies conducted by other authors: below or above legislation standards[15, 17-19].

The quantification of the residual levels of nitrites and nitrates consists in reducing nitrite and nitrate with spongy cadmium in alkaline medium followed by diazotization of nitrites with sulfanilic acid and mating with alpha-naphthylamine hydrochloride in acid medium to form the pink-colored alpha-naphthylamino-p-azobenzene-p-sulfonic. The resulting product was quantified by spectrophotometry at 540 nm[20] using a Perkin Elmer Lambda 25 spectrophotometer.

The pH of the samples was determined using a Digimed DM-22 pH meter; and water activity was determined using an Aqualab Lite meter – Aw Analyzer – Decagom (BrasEq).

Descriptive statistics (mean and standard deviation) was used for quantitative variables with normal distribution; and median and interquartile range were used for quantitative variables with asymmetrical distribution. Qualitative variables are described as absolute and relative frequencies. Comparisons of these bicategorical variables between the brands analyzed were performed using the chi-squared test and the comparison of quantitative variables between the brands was performed using Anova. The analysis of statistical data was performed using the Statistical Package for the Social Sciences (SPSS), version 18.0, and a significance level of $p<0.05$.

RESULTS

Less than half of the samples – 40.3% (29) – presented nitrite levels above the recommended by the legislation, while half of the samples 50% (36) showed nitrate levels above the standard levels and 100% (72) of the samples presented pH and water activity within the recommended standards for all the sausages analyzed.

Descriptive statistics for the total sample of sausages analyzed are presented in Table I. According to the current law, Ordinance No. 1.004, of 11 December 1998, of MAPA[15], the mean nitrite of the samples of sausages analyzed (112.13 ±89.39 mg/kg) was significantly lower than the value established by the legislation, which is 150 mg/kg ($p=0.001$); in addition, the mean nitrate (389.01 ±280.84 mg/kg) was significantly higher than the recommended value, which is 300 mg/kg ($p=0.009$). The mean pH value was also significantly higher (6.33 ±0.32) than the value established for low-acid foods ($p <0.001$), and the mean water activity (0.91 ±0.01) was significantly lower ($p<0.001$).

When comparing the sausage brands as for the mean nitrite, nitrate, pH and water activity levels, a significant difference was found for all variables, as shown in Table II.

The sausage brand C was the one with the highest levels of nitrate and nitrite and the highest pH. Although significant, the difference in water activity between the brands was very little.

When comparing the brands as for the percentage of samples with nitrite and nitrate levels within the standards recommended by the Brazilian legislation, a significant difference was observed, as shown in Table III.

Table I - Descriptive statistics of the variables analyzed in the sample of sausages. Lajeado, 2015.

Variable	Descriptive Statistics
Nitrate (mg/kg)	335.40 (121.31 - 631.81)
Nitrite (mg/kg)	62.12 (34.71 - 178.98)
pH	6.33 ± 0.32
Water activity	0.91 ± 0.01

Data are described as mean ± standard deviation or median (P25 - P75). *pH: potential of hydrogen

Table II - Comparison between sausage brands as for the levels of nitrate, nitrite, potential of hydrogen (pH) and water activity (Aw). Lajeado, 2015.

Brand	Nitrate (mg/kg)	Nitrite (mg/kg)	pH	Aw
A	92.7± 15.9	39.5± 8.7	6.3±0.2	0.91±0.01
B	124.4±6.9	36.7±2.6	6.1±0.2	0.91±0.01
C	641.2±39.4	269.5±12.4	6.5±0.2	0.91±0.01
D	140.8±17.8	13.7±5.2	5.8±0.2	0.92±0.01
E	650.3 ±139.4	171.8 ±28.7	6.8±0.1	0.92±0.01
F	723.4 ±128.6	151.6 ±58.4	6.3±0.2	0.91±0.01
G	306.9±241.3	83.9±68.3	6.4±0.2	0.91±0.01
H	432.4±270.1	130.3±60.4	6.3±0.3	0.91±0.01
p value*	<0.001	<0.001	<0.001	0.004

*Anova test. Data are described as mean ± standard deviation.

Table III - Comparison between sausage brands as for compliance with legislative limits of nitrite and nitrate. Lajeado, 2015.

Brands	Nitrate	Nitrite
A	9 (100%)	9 (100%)
B	9 (100%)	9 (100%)
C	0 (0%)	0 (0%)
D	9 (100%)	9 (100%)
E	0 (0%)	3 (33.3%)
F	0 (0%)	3 (33.3%)
G	6 (66.7%)	6 (66.7%)
H	3 (33.3%)	4 (44.4%)
p value*	<0.001	<0.001

*Chi-squared test. Data are described as absolute and relative frequencies.

DISCUSSION

As the levels of nitrite and nitrate in some brands analyzed in the present study are significantly higher than the legislative limits, food security and health promotion are compromised due to the health risks caused by the toxic effects of these substances – depending on the amount consumed and the body susceptibility[14].

In the present study, the mean nitrite level of the samples was significantly lower and the mean nitrate level was significantly higher than the legislative limits, highlighting a non-compliance with the law by the companies studied. These results are worrying since the consumption of these substances above the recommended levels may result in health problems such as cancer and methemoglobinemia[21-23]. Thus, the need for constant monitoring of manufacturers of meat products with added nitrite and nitrate by public health agencies should be highlighted in order to guide the manufacturer and monitor the use of these additives[7].

The mean pH value in the present study was also significantly higher (6.33 ±0.32) than the value established

for low-acid foods; also, the mean water activity was 0.91 ±0.01, a value that is significantly lower than the ones reported in other studies[18].

A study on sausages[24] found that 30.3% and 69.7% of the samples presented nitrite and nitrate levels, respectively, above the legislative limits, a result that is different from those observed in another study on sausages in which all the samples were within the legislative limits of nitrite and nitrate[25]. In another study on sausages and mortadellas, all the samples analyzed were within the legislative limits; however, the samples of bacon, which is also considered a type of encased meat, presented mean values above the limits set for nitrite and nitrate[13].

Regarding nitrite levels in hot dog sausages marketed in the metropolitan region of Recife and undergoing federal inspection, both group A (from Southern Brazil) and group B (from Northeastern Brazil) had all the samples within the recommended parameters. As for the samples of local industries in Recife – unknown brands and inspection and obtained in street markets (group C) – 67% of the samples had residual nitrite >150 mg/kg, which corresponds to 18% of all samples analyzed. As for nitrate levels, 17% of the samples in group A, 67% in group B and 83% in group C were above the legislative limits[2].

When nitrite levels are high, such as in brands C, E and F of the present study, there may be great difficulty in the oxygenation process in the body of individuals, leading to the development of symptoms such as cyanosis, nausea, vomiting, abdominal pain and collapse[26]. Studies examined the relationship between the consumption of food with nitrites and N-nitrosamines and the onset of stomach and esophageal cancer as well as a positive association between the consumption of processed meat and the incidence of cancer[21,22]. The present study also found high levels of nitrate in the brands C, E, F, G and H – a worrying result since nitrate undergoes enzymatic and/or microbial reduction to nitrite[27,11].

In newborns, a high intake of nitrate has been associated with methemoglobinemia or blue baby syndrome. The conversion of nitrate to nitrite is enhanced by the low stomach acidity in children, which promotes the growth of micro-organisms capable of reducing nitrate to nitrite, a situation that can be influenced by breastfeeding[23]. Healthy adults are able to consume larger amounts of nitrate with smaller effects[28]; therefore, the nitrate toxicity is lower than that of nitrite. A study shows that 0.15 mg/kg of nitrate does not alter the amount of methaemoglobin; however, exceeding 15 g/kg can be fatal. With regard to nitrite, just 32 mg/kg can be lethal[29].

For being an ultra-processed food, sausage consumption is not recommended due to its unfavorable nutritional composition and the impact that their forms of production, distribution, marketing and consumption have on culture, social life and on the environment, especially among children[30].

As for pH and water activity analysis, all the samples were in accordance with the recommendations, and the mean pH found in the present study was similar to that found in other studies[31,25] assessing hot dog sausages. The pH oscillation is probably due to the possible presence of lactic acid bacteria, which are important spoilage microorganisms in cooked encased meats, and/or because of the characteristics of the antioxidant and stabilizer used in the sausage processing[31,25]. The mean water activity found in the brands analyzed in the present study was similar to the results of a study that found a value of 0.88[31]; however, other studies found water activity levels ranging from 0.93 to 0.94, both for vacuum-packed and bulk-packed samples[18], with the highest mean of 0.97[25]. Higher levels of water activity in the sausages derive from their composition, in which the excessive incorporation of mechanically separated meat and polyphosphates results in a product with increased juiciness and tenderness[1].

Factors such as water activity, pH and the chemical composition of foods indicate the type of microbial spoilage in the product; thus, the results of the present study showed an adequacy of all samples analyzed in relation to pH and water activity, demonstrating good chances of these samples presenting an adequate microbial growth[32].

From a total of eight brands analyzed in the present study, only three presented fully satisfactory conditions in relation to the addition of nitrate and nitrite. The inspection by the competent bodies is of fundamental importance since consumers are susceptible to the risks inherent to the intake of processed foods in unfavorable conditions regarding the additives used[7]. As described throughout the present study, the use of these salts above the maximum permitted levels can cause serious risks to human health due to the possibility of manifestations of acute and chronic toxic effects[2].

It is the consumer's right to have access to safe food free of risks to health; and if they are not safe, as shown by the present study, they represent a potential risk to the health of those who consume them given the failure to comply with the legislation during their production.

The limitations of the present study may be related to the number of brands selected for analysis; the study included the brands marketed in the region of Vale do Taquari, located in Rio Grande do Sul; therefore, they may not be the same brands marketed in other regions. However, as they undergo federal inspection, they can be consumed in any region of Brazil.

CONCLUSION

It was concluded that all the samples complied with legislative limits of potential of hydrogen (pH) and water activity (Aw). Of the eight brands analyzed in the present study, only three presented fully satisfactory conditions in relation to the addition of nitrate and nitrite. The mean nitrite and Aw were significantly lower, and the mean nitrate and pH were significantly higher than the values established by the references used. However, nitrate and nitrite levels means were significantly higher than the legislative limits in five of the brands analyzed, showing that there is no standardization regarding the addition of these additives by the companies.

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