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Quantification of Nitrate and Nitrite in Sausage

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Abstract

Sausage is a type of meat product usually made from pork, beef or poultry. Nitrite and nitrate are preservatives used in meat products (sausages) for their effects on microbial and chemical properties and sensory quality. Cancer risk is one of the most important nitrite issues because it is the reaction of Nitric Acid with secondary amines that create nitrosamines.The aim of this study was to determine the amount of nitrites and nitrates in different types of sausages produced by Albanian companies and imported. A total of 7 samples were randomly purchased from the market and chemical properties such as water activity, moisture, and pH were evaluated. The determination of the level of nitrites and nitrates was done by spectrophotometric method.The concentration of nitrites and nitrates in different types of analysed sausage was between 19-189 ppm and 24-261 ppm, respectively. While 42.8 % and 14.3 % of the samples presented nitrite and nitrate levels, respectively, significantly above what is recommended by the legislation.The results of this study show that the samples were found with nitrite and nitrate residuse higher than the limit allowed in meat products, so, it is important that the subjects of official control carry out routine surveillance of sausages that are sold in markets, thus avoiding health problems for consumers.

Keywords: nitrate; nitrite; sausage.

1. Introduction

Sausages are an important gastronomic and nutritional heritage, which play an important role in people's daily life [4]. The trend for healthy lifestyles and, accordingly, healthy eating is combined in today's society with time savings and the purchase of ready meals and semi-finished products. Traditionally, sausage products are purchased. They occupy a large proportion in human nutrition [9].

The process of preserving meats by stuffing salted, chopped meats flavoured with spices into animal casings dates back thousands of years, to the ancient Greeks and Romans, and earlier. The word "sausage" is derived from the Latin word "salsus", which means salted, or preserved by salting. Sausages and sausage products have since evolved into a wide variety of flavours, textures, and shapes resulting from variations in ingredients and manufacturing processes [7].

Sausage manufacturing consists of grinding, mincing or chopping the muscle tissue and other organs and blending them with: fat, salts, seasonings (herbs and spices) and, when necessary, with binders or extenders [2].

Substances of chemical, plant and animal origin are also used as an ingredients. These substances are called additives and play functional role in assuring safe for consumers and in improving processing technology and some sensory quality, as instance for improving: taste, flavour, colour, water biding capacity, texture, etc. Ingredients of plant origin like spices are mainly used to improve taste and flavour of meat products. Ingredients of chemical origin, like: nitrites, salts, antioxidants, preservatives, etc., are mainly used in small limited amounts to improve product's structure, water biding, shelf-live and sometimes even flavour and colour. Ingredients of animal origin are mainly used to improve water biding and to avoid fat split during heat treatment [6]. The chemical additives, especially sodium nitrite, play a major role in improving colour, taste, shelf-life, and preventing the growth of *Clostridium botulinum*, however, they can also have disadvantages such as the formation of nitrosamines and methemoglobinemia [1].

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In terms of conservation, these compounds are of great importance for the technological area of food. On the other hand, their intake is controversial as to their harmful effects on human health, especially in relation to the development and progression of cancers [8]. In order to regulate the application of nitrite and nitrate in meat or meat products, many countries have established directives and regulations [3].

The purpose of this study is to investigate the levels of nitrites and nitrates in sausages sold in Tirana, produced by Albanian and imported companies, during June 2022, compared to the standards.

2. Material and Methods

2.1. Sample collection and preparation

The study was based on 4 samples of sausages obtained from Albanian companies and 3 samples of imported sausages, collected in markets in Tirana during the month of June 2022. Before performing the chemical analysis, each samples was ground and homogenized by a laboratory meat blender. Sausage samples of each brand were analysed for moisture, water activity and pH. Also, the samples were analysed to determine the concentrations of nitrites and nitrates by the spectrophotometric method.

2.2. Analytical techniques

Chemical parameters as water activity and pH were determined with relevant apparatus. The moisture content was determined thermogravimetrically method, by drying well homogenized samples respectively at 105 ºC to constant weight. Percentage of moisture content of sausages samples was calculated based on weight lost.

The quantification of nitrites and nitrates in the sausage samples was carried out by spectrophotometric methodology, based on the reduction of nitrates to nitrites in the presence of zinc powder. The nitrite (nitrite present plus reduced nitrate) is determined by Griess reaction to form a highly coloured azo dye that is measured at 540 nm. The nitrite present in the sample is determined by analysing without the reduction step. The nitrate is calculated as the difference between the total nitrite content after reduction and the initial nitrite concentration.

Initially was prepared the calibration graph for nitrite and nitrate, in the concentration range 0.2-1.2 mg/l. The samples were prepared: firstly were homogenized with a grinding device and extracted with hot water. They were then clarified with Carrez I & II solution and were filtered. For the determination of nitrites in the filtered sample, the reagents for the development of the Griess reaction were added. After incubation, the absorbance was measured at 540 nm with a spectrophotometer. For the determination of nitrates, the reduction of nitrate to nitrite is carried out, for this zinc powder is added to the filtered sample. The reagents for the developing of the Griess reaction were then added. After incubation, the absorbance was measured at 540 nm with a spectrophotometer. The concentration of nitrites and nitrates was calculated.

3. Results and Discussion

3.1. Chemical parameters

Table 1 shows the analyzed chemical parameters such as: water activity (Aw), pH and moisture for four samples of sausage produced in Albania and three imported sausages. The data are the average values of analysed parameters. Water activity in meat and meat products becomes more important, since the aw-value affects different chemical reactions in the product as well as the surviving and the resistance of microorganisms. Based on these results, the water activity of the analysed sausage samples produced by the Albanian companies is above 0.90, only one sample that is 0.93. Also, the Aw for the imported sausages is 0.91, except for one sample that is 0.81. The water activity of sausages analysed varying from 0.81 to 0.93 and it is lower due to higher salt content. Lower water activity improves the shelf life and safety meat products, because they are more stable to microorganisms that can cause spoilage or food poisoning.

The pH value in meat products (sausage) depends on various factors (type of meat, fermentation, other ingredients, etc.) but it is an important parameter because it affects the characteristics of the product (such as colour, taste, shelf life). The pH values were 6.13-6.30 for sausages produced in Albania and 5.79-6.07 for imported sausages, we see that the pH range is generally from 6.1 to 6.3 for both types of local and imported sausage products, only one sample of imported sausage has a lower pH value of 5.8.

Moisture content is also an important parameter, but there are large variations in sausages (processed animal foods), above all it is directly related to fat content, because fatty tissues is added as an ingredient during preparation. It is noted that in 57.1 % of the analysed

samples the moisture content is from 59.52-65.20 %, while one sample from local products has 45.63 % moisture because this is a semi-dry sausage, while two samples (one from Albanian companies and one from imported sausages) have the lowest moisture of 20,66 and 29.00 % because they were dry sausages.
From data we see that there is no relationship between water activity and moisture content.

Table 1. Physicochemical properties of analysed sausage

	a_w		pH		Moisture %	
No. sample	**Albanian producer**	**Imported**	**Albanian producer**	**Imported**	**Albanian producer**	**Imported**
1	0.90	0.91	6.16	6.07	59.52	61.42
2	0.90	0.91	6.22	6.09	45.63	63.75
3	0.91	0.81	6.30	5.79	20.66	29.00
4	0.93		6.13		65.20	

3.2. Determination of nitrites and nitrates

The use of nitrates and nitrites as meat curing agents is defined by the Food Additives Regulations, but prevents the use of overdose by food processors as the amounts of quantities in the final products are limited according to the Regulation (EC) No 1333/2008 [9]. For this reason, another objective of our study was the quantitative determination of nitrite and nitrate levels in sausage by the spectrophotometric method. Calibration curves were first constructed for nitrite (NO_2^-) and nitrate (NO_3^-) and then the concentration of nitrite and nitrate for each sample was calculated and shown in Table 2.

Table 2. The concentration of nitrites and nitrates in the analysed sausage from the Albanian companies and imported

	Albanian producer		**Imported**	
No. sample	**Conc. NO_2^- mg/kg**	**Conc. NO_3^- mg/kg**	**Conc. NO_2^- mg/kg**	**Conc. NO_3^- mg/kg**
1	148.25	23.97	104.91	51.51
2	20.29	260.79	189.13	163.10
3	23.19	101.29	18.70	80.70
4	60.27	91.63		

Referring to the data, it is noted that the levels of nitrites and nitrates in the samples produced in Albania were respectively from 20.29-148.25 mg/kg and 23.97-260.79 mg/kg . Also, the concentration of nitrites and nitrates in imported samples were from 18.70-189.13 mg/kg and 51.51-163.10 mg/kg respectively.

From the figure 1, it can be seen that the imported samples present higher nitrite concentration values than locally produced samples.

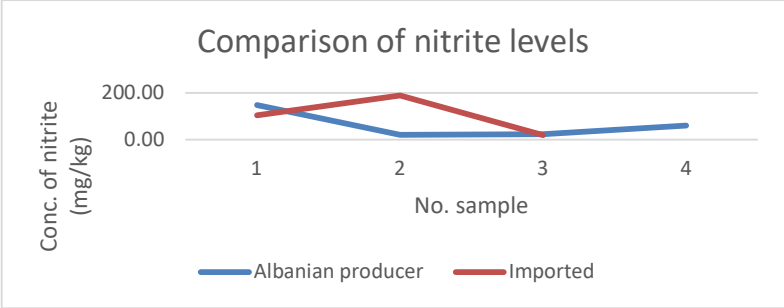

Figure 1. Comparison of nitrite levels in analysed samples

Regarding the concentration of nitrates in figure 2, we can see that the sausage samples from the Albania companies have higher nitrate concentration values than the imported samples.

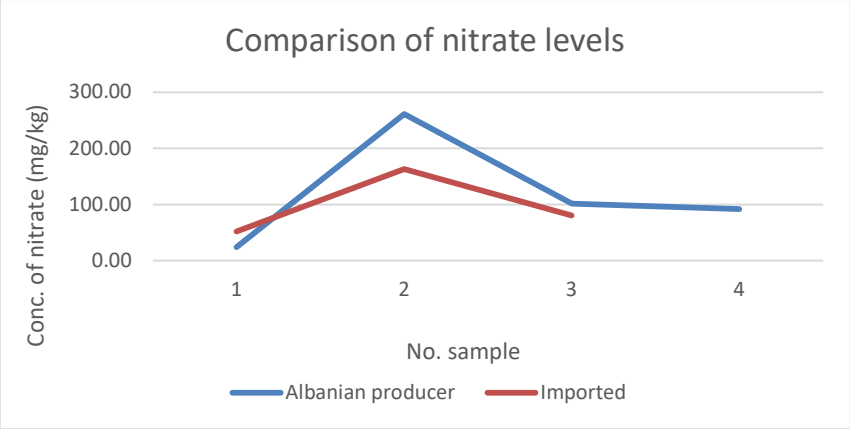

Figure 2. Comparison of nitrate levels in analysed samples

The results obtained showed that in 57 % of the analysed samples the nitrate concentrations were higher than the nitrite concentration. In three samples (43 %), it happens that the concentration of nitrite is higher than nitrate, and they are the samples in which the content of nitrite is higher.

Analytical data show that: in 42.8 % (three samples: one produced in Albania and two imported) of the sausage samples, the nitrite content was above the maximum allowed concentration established by European Commission Regulations in meat products, for nitrites 100 mg/kg; in 14.3 % (one sample produced in Albania) of the sausage samples, the nitrate content was above the maximum allowed concentration established by European Commission Regulations in meat products, for nitrates 250 mg/kg.

4. Conclusions

Based on results of chemical properties such as: pH, water activity and moisture, we conclude that there are no significant differences between sausages produced in Albania and imported ones. But the nitrite content is lower in sausages produced in Albania than imported and the nitrate content is lower in the imported samples than those produced in Albania.

Nitrite levels in analysed sausages are lower than nitrate levels. In analysed samples, it results that the concentration of nitrites in 42.8 % of the sausage samples and the concentration of nitrates in 14.3 % of the analysed sausages is higher than the maximum concentrations allowed by the Regulation of Food Additives.

The results indicate that the concentrations of nitrates and nitrites in the final products (sausages) should be

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limited and monitored, so as not to cause a risk to human health.

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