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PHOSPHATE AND NITRITE - THE MOST COMMONLY USED ADDITIVES IN THE MEAT INDUSTRY

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Summary

Food additive is any substance that, regardless of its nutritional value, is not used as food, nor is a characteristic ingredient of food, but is added to food for technological reasons during production, processing, preparation, packaging, transport or storage. Thus, through its intermediate products, it becomes or can become a food ingredient. The most commonly used additives are nitrites and phosphates, due to their multiple role in the meat industry. According to the Regulation on food additives (Official Gazette of the Republic of Srpska, 96/20), the maximum accepted amount of phosphoric acid and phosphate (E 338 - E 452), which can be added to meat products individually or in combination (expressed as P_2O_5), is 5 g/kg. Many studies have confirmed the connection between a high intake of phosphate additives and various diseases in humans. Nitrites are preservatives that are added to meat products in order to improve the quality, durability and safety of the products, and they must not be added in an amount higher than 150 mg/kg in most meat products. Due to the possibility, under certain conditions, of the formation of carcinogenic nitrosamines in meat products, there is a potential danger to human health. The aim of the study was to determine the content of residual nitrite and added phosphates (expressed as phosphorus pentoxide P_2O_5) in meat products originating from the market of Republika Srpska (Bosnia and Herzegovina) and compliance with regulatory requirements. The results showed that the determined amounts of residual nitrite and added phosphates were within the acceptable limits for addition to different categories and types of meat products according to current regulations. This confirms that the application of nitrite and phosphate is adequate and that the product is healthy. In order to protect the health of consumers, it is important to continuously monitor the use of phosphates and nitrites in the meat industry.

Key words: additives, meat products, nitrites, phosphates, human health risk.

INTRODUCTION

Meat and meat products are important sources of energy and nutrients, and have an important role in a balanced diet of human population. Meat and meat products have a significant role in human nutrition, which is evidenced by the long history of their consumption, since it may be traced back about 3 million years (Specht, 2019; McKenna, 2019).

Numerous additives are used in the production of meat products. A food additive is any substance that, regardless of its nutritional value, is not used as food, nor is a characteristic ingredient of food, but is added to food for technological reasons during production, processing, preparation, packaging, transport or storage, so that directly or indirectly through its intermediate products it becomes or can become a food ingredient. Thanks to their multiple role in the meat industry, the most commonly used additives are nitrites and phosphates (Milešević et al., 2022).

According to the Regulation on food additives (Regulation, 2020), the maximum accepted amount of phosphoric acid and phosphate (E 338 - E 452), which can be added to meat products individually or in combination (expressed as P_2O_5), is 5 g/kg. Since meat contains natural phosphorus, the phosphate content in meat products is expressed as the total phosphates content which includes added and natural phosphorus. In the processing of meat and meat products, phosphates are necessary for several reasons such as: increasing the pH value, increasing the water retention capacity (the structure of the muscle protein is open), higher yields and stabilizing the meat emulsion, reducing weight loss during cooking, improving the texture and sensory properties (tenderness, juiciness, color, taste), extension of shelf life, etc. (Knipe, 2003; Lampila and Godber, 2002; Molins, 1991; Polak et al., 2017). However, the use of phosphates in the meat industry is under serious supervision due to the growing interest in healthier foods and the health risks that are related to consumption of synthetic phosphates. These “free” (inorganically bound) phosphates are efficiently absorbed in the gastrointestinal tract (Glorieux et al., 2017). High intake of phosphate additives is associated with mortality caused by cardiovascular diseases (Ritz et al., 2012). Widespread use of phosphate additives is also associated with calcium and phosphorus imbalance (Ling et al., 2020).

Nitrites are preservatives that are added to meat products to improve quality, durability and safety, and in most meat products they must not be added in an amount higher than 150 mg/kg (Regulation, 2020). They are most often used for brining in the meat industry, and they can also affect human health. Due to carcinogenic

nitrosamines that can be formed in meat products under certain conditions (low pH value, high temperature), there is a potential risk for human health (Vuković, 2012; IARC, 2010; IARC, 2018). By adding nitrite, a better taste and aroma of the product is achieved, the red-pink color of the meat is formed, as well as the appropriate sustainability and safety of the product thanks to their antimicrobial and antioxidant action. The antimicrobial effect is based on the activity of undissociated nitric acid, which is formed from nitrite, and has an inhibitory effect against *Clostridium botulinum*, which is known as the cause of botulism (Marco et al., 2006; Sebranek and Bacus, 2007; Ferysiuk and Wójciak, 2020).

The aim of the study was to determine the content of residual nitrite and added phosphates (expressed as phosphorus pentoxide P_2O_5) in meat products originating from the market of Republika Srpska (Bosnia and Herzegovina) and compliance with regulatory requirements.

MATERIALS AND METHODS

The study is based on the determination of the content of residual nitrite (nitrites) and added phosphates (phosphates) expressed as phosphorus pentoxide (P_2O_5) in various meat products, systematized into several categories and subgroups according to the current Regulation (2015).

In the period from August 2022 to April 2023, 120 samples of meat products were tested, of which nitrite content was tested in 115 samples, and phosphate content in 92 samples. The samples came from the territory of Republika Srpska (Bosnia and Herzegovina).

Upon receiving the samples in the laboratory, they were homogenized, stored in a hermetically sealed container at 4°C and analyzed within 24 hours from homogenization.

The test was conducted using the following methods:

- content of total phosphates according to BAS ISO 13730 (ISBIH, 2022),
- protein content according to BAS ISO 937 (ISBIH, 2007a)
- nitrite content according to BAS ISO 2918 method (ISBIH, 2007b).

The determination of added phosphate (phosphate additives) was done according to a mathematical formula using the conversion of phosphorus expressed as phosphorus pentoxide content (P_2O_5). This formula provides the values for content of total phosphates and proteins (FAO/WHO, 2019a; FAO/WHO, 2019b; ABH, 2015):

$$\text{Natural phosphate (mg/kg } P_2O_5) = 250 \times \text{amount of protein (\%)} \quad (1)$$

$$\text{Total phosphate (mg/kg } P_2O_5) - \text{natural phosphate (mg/kg } P_2O) = \text{added phosphates (mg/kg } P_2O_5)$$

The results obtained in this study were statistically processed. Basic descriptive statistical parameters were used. Results were presented in tables.

RESULTS AND DISCUSSION

Assessment of compliance with the requirements of the Regulation on food additives (Regulation, 2020) was carried out based on the content of added phosphates and residual nitrite according to Table 1.

Table 1 Accepted amounts of residual nitrites and added phosphates that can be added to meat products (Regulation, 2020)

Parameter	Regulation requirement
Nitrites (Na-nitrite)	150 mg kg ⁻¹
Phosphates (expressed as P ₂ O ₅)	5 g kg ⁻¹

The results of analyzes of the amount of protein (%), total phosphates (g kg⁻¹), added phosphates (g kg⁻¹) and nitrites are shown, by groups, in Tables 2, 3, 4 and in Figures 1 and 2.

Table 2 shows the results of the nitrite concentrations (mg kg⁻¹) in meat products, by group, according to the current Regulation (2015). Nitrites were not quantified in 29% of the tested samples (Figure 1), and those samples are chopped shaped meat *cevap* and fermented dry sausages. In minced meat-*cevap*, this result is expected because the use of nitrites is not allowed in minced meat.

Higher concentrations of nitrite were detected in thermally processed products, and less in dry products, which is in agreement with the results of the Pećanac and Sladojević (2022). The highest value of sodium nitrite was found in coarse ground sausages and was 68 mg kg⁻¹, while in fine ground sausages the maximum amount was 46 mg kg⁻¹. Pećanac and Sladojević (2022) examined the nitrite content in 576 meat products and determined 106 mg kg⁻¹ of nitrite in coarse ground sausage, and 88 mg kg⁻¹ in fine ground sausage, which is significantly higher than the results obtained in this study.

Kovačević et al. (2016) determined the amount of sodium nitrite in different meat products as follows: 7±4 mg kg⁻¹ in dry sausage, 24±16 mg kg⁻¹ in thermally processed sausage, 37±23 mg kg⁻¹ in dry meat product and 42±21 mg kg⁻¹ in the semi-dry meat product. Our results are in accordance with the data given for fermented dry sausages, significantly lower for dry and semi- dry meat products, and higher for heat-treated sausages.

Pavlinić Prokurica et al. (2010) indicate that nitrates can only be found in those meat products to which nitrites have been previously added. Through the oxidation reaction, about 20% of the added nitrites change to nitrates two hours after processing and this process continues during storage. The conversion of nitrates to nitrites in dry meat products is provoked by the addition of ascorbate or its isomer erythorbate, the addition of sodium ascorbate as a preservative. However, nitrates are also produced without previously mentioned addition, but in smaller quantities, in other meat products.

Table 2 Results of nitrite testing (mg kg^{-1}) in meat products from the market of Republika Srpska shown by subgroups

Subgroup of meat products	Number of samples	n < LOQ	Mean \pm SD mg kg^{-1}	Minimally detected mg kg^{-1}	Maximum detected mg kg^{-1}
Fine chopped sausages	10	-	34.3 \pm 17.0	7	46
Coarse chopped sausages	26	-	31.7 \pm 20.5	5	68
Dry fermented sausages	19	9	5.1 \pm 1.8	2	8
Minced meat cans – canned meat	14	5	8.3 \pm 9.2	3	32
Pate	10	2	16.0 \pm 15.4	2	37
Semi-dry meat products	3	-	24.7 \pm 2.0	7	46
Dry meat products	4	1	11.0 \pm 9.6	4	25
Heat-treated meat sausages in pieces	2	-	25.5 \pm 0.71	25	26
Semi-dry bacon	5	1	18.5 \pm 22.69	4	52
Chopped shaped meat <i>cevap</i>	15	15	-	-	-
Pressed ham in wrapper	7	-	29.7 \pm 19.47	6	58

n: number of samples in which nitrites were not quantified; LOQ: limit of quantification is 2 mg kg^{-1} . Figure 1 shows the number of samples in which nitrites were not quantified ($\text{LOQ} < 2$).

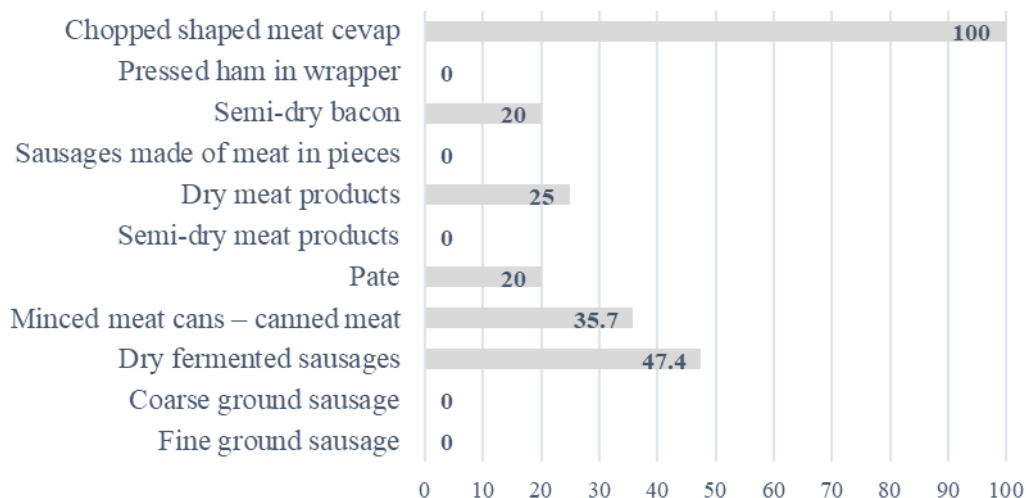
Nitrites were not determined (%)

Figure 1 Percent of samples in which nitrites were not quantified (LOQ < 2 mg kg⁻¹)

In dry sausages, the highest nitrite content is detected in beef tea sausage (8 mg kg⁻¹), and the lowest in pork tea sausage (2 mg kg⁻¹). In fine chopped smoked sausages, the highest nitrite content was determined in chicken special sausage (46 mg kg⁻¹), and the lowest in the similar chicken meat product (7 mg kg⁻¹). In coarse ground sausages, the highest content is detected in the barbecue sausage, and the lowest in the similar pork product. In semi-dry meat products, the highest nitrite content is 46 mg kg⁻¹ in smoked neck, and the lowest is 7 mg kg⁻¹ in smoked sirloin. In dry cured meat products, the highest (25 mg kg⁻¹) and lowest nitrite content (7 mg kg⁻¹) was found in dry sirloin. The highest nitrite content (37 mg kg⁻¹) was found in chicken pate.

Table 3 shows the total phosphates, proteins and added phosphates content by product subgroups, and Table 4 shows the minimum and maximum values of the content of added phosphate.

Table 3 Protein, total and added phosphates content (g kg⁻¹) in meat products by subgroups

Subgroup of meat products	Protein %	Min %	Max %	Total phosphate kg ⁻¹	Min g kg ⁻¹	Max g kg ⁻¹	Added phosphate g kg ⁻¹
Fine chopped sausages (n=10)	11.22±1.04	9.61	12.11	4.44±0.32	4.15	5.04	1.64±0.22
Coarse chopped sausages (n=22)	15.00±1.95	13.42	18.21	5.70±0.62	4.66	6.32	1.94±0.53
Dry fermented sausages (n=19)	23.31±2.90	18.55	29.19	5.50±0.83	3.94	7.10	<0.10
Minced meat cans – canned meat (n=13)	12.29±5.00	10.55	14.04	5.00±0.69	4.12	6.17	1.90±0.84
Pate (n=5)	9.82±0.903	9.05	11.39	2.86±0.69	2.32	3.94	<0.10
Semi-dry meat products (n=3)	19.36±3.315	15.63	21.96	5.72±0.30	5.37	5.91	0.92±1.04
Dry meat products (n=3)	34.44±2.89	31.11	36.28	7.18±0.53	6.58	7.57	<0.10
Heat-treated meat sausages in pieces (n=2)	10.63±1.94	9.25	12.0	4.64±0.38	4.37	4.91	1.99±0.87
Semi-dry bacon (n=5)	17.52±4.35	10.30	21.84	4.20±0.82	3.42	5.37	0.97±0.16
Pressed ham in wrapper (n=5)	15.00±1.95	13.42	18.21	5.70±0.62	4.66	6.32	1.94±0.54
Meat dishes (n=5)	8.10±1.42	7.41	10.64	1.78±0.26	1.33	1.95	<0.10

LOQ: limit of quantification 0.10 mg kg⁻¹; n: number of samples with nitrite content < LOQ

Added phosphates were not quantified in 39% of samples (< 0.10 g kg⁻¹). In all samples of dry fermented sausages, pate, dry cured meat products and meat dishes, added phosphates are below the limit of quantification. Also, in four out of five bacon samples, added phosphates were not quantified. In the meat industry, phosphates are used in amounts of 0.05 to 0.5%. It is important to emphasize that in dry meat products with a high protein content, in which polyphosphates are not even used, a high natural level of phosphorus was determined during the analysis

of total phosphorus (Marušić et al., 2012). In the study done by Milešević et al. (2022), the highest average and highest content of total phosphorus was found in dry meat products ($6.12 \pm 1.33 \text{ g kg}^{-1}$ and 10.64 g kg^{-1} , respectively), which is in agreement with our results. Also, our results for total phosphates in canned minced meat – canned meat ($5.00 \pm 0.69 \text{ g kg}^{-1}$) are very similar to the results of Milešević et al. (2022), who determined $5.79 \pm 1.01 \text{ g kg}^{-1}$ of total phosphates.

In the subgroup of fine chopped sausages, the highest amount of added phosphates was found in the similar chicken meat product (2.01 g kg^{-1}), while in coarse chopped sausages, the maximum amount was found in Carniolan sausage (2.48 g kg^{-1}). A maximum of 2.12 g kg^{-1} of added phosphates was determined in the smoked neck from the subgroup of semi-dry meat products.

Pećanac and Brenjo (2022) determined phosphates in 247 meat products. They did not determine added phosphates ($<0.10 \text{ g kg}^{-1}$) in 26% of products. The highest average value of phosphate ($3.09 \pm 1.097 \text{ g kg}^{-1}$), as well as the highest determined content, was found in sausages made of meat in pieces, which is in accordance with the results of our study. However, regarding the highest determined phosphate content, the results do not agree with the results of Pećanac and Brenjo (2022). The same authors determined the lowest phosphate content (0.26 g kg^{-1}) in coarse chopped sausages, while in our study the lowest value was determined in semi-dry meat products.

Table 4 Minimum and maximum values for the content of added phosphates in meat products

Category	min g kg ⁻¹	max g kg ⁻¹
Fine chopped sausages	1.32	2.01
Coarse chopped sausages	1.30	2.48
Dry fermented sausages	<0.10	<0.10
Minced meat cans – canned meat	0.62	3.32
Pate	<0.10	<0.10
Semi-dry meat products	0.24	2.12
Dry meat products	<0.10	<0.10
Meat sausages in pieces	1.37	2.6
Semi-dry bacon	<0.10	1.08
Pressed ham in wrapper	1.3	2.48
Meat dishes	<0.10	<0.10

The highest content of phosphate (3.32 g kg^{-1}) was determined in minced meat cans – canned meat, and the lowest in semi-dry meat products (0.24 g kg^{-1}), which is not in accordance with the results of Pećanac and Brenjo (2022). The mentioned authors determined the lowest average phosphate value in pâtés ($0.911 \pm 0.485 \text{ g kg}^{-1}$).

Pećanac and Brenjo (2022) determined the highest average value of added phosphates ($3.09 \pm 1.097 \text{ g kg}^{-1}$), as well as the highest content of phosphates (4.69 g kg^{-1}), in sausages made from meat in pieces, and the lowest in Tyrolean sausage (0.26 g kg^{-1}).

Before using any type of meat for the preparation of meat products, it is necessary to know the amount of phosphorus in the meat or chopped meat for shaping, so that the added synthetic phosphates and polyphosphates do not exceed the accepted limit (Prica et al., 2015).

CONCLUSION

The conducted study determined that the amounts of residual nitrite and added phosphates and showed that those are within the limits defined by current regulations meaning that they are allowed for addition to different categories and types of meat products. This confirms the adequate application of nitrite and phosphate and the safety of the product.

Higher concentrations of nitrites were determined in heat-treated products, and less in dry heat-untreated products. Nitrites were not quantified in 29% of the tested meat products, which mainly belong to dry fermented sausages and *cevap* (shaped minced meat).

Added phosphates were not quantified in 39% of samples ($< 0.10 \text{ g kg}^{-1}$), which belong to dry fermented sausages, pates, dry meat products, meat dishes and dry bacon, which was expected considering that in the production of some meat products the use of phosphate is not technologically justified.

In order to protect the health of consumers, it is important to continuously monitor the use of phosphates and nitrites in the meat industry.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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