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Original scientific paper

Phosphate additives in meat products: analytical determination and interpretation of results

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A B S T R A C T

Analytical determination of phosphate additives in food presents some difficulties that are not only related to the applied analytical technique, but also to the ingredients used in production. Meat, by itself, contains organically bound phosphorus, but also inorganic phosphates. Even water used in production can contain certain amounts of inorganic phosphates. That is why a measurable quantity of phosphate exists in products even without the addition of permitted phosphate additives.

By applying ion chromatography to determine the total inorganic phosphate content in meat products, it was found that the greatest number of products that did not comply with the regulation were those in which phosphate additives are seldomly added. About one fifth of analysed fermented sausages and about one quarter of dried meat products contained phosphates at levels that exceeded the maximum permitted level, expressed as P2O5. The main reason may be that these products go through a drying phase during production, whereby, with the decrease in the amount of water in them, the share of other ingredients, as well as phosphate, increases.

1. Introduction

In humans and animals, phosphorus is involved in various physiological processes, such as acid-base balance regulation, it is a crucial part of the cell's structural and energy cycle, is a regulating and signalling component and has a significant role in the mineralization of teeth and bones. The versatility of the physiological aspects of phosphorus made it an essential nutrient with serious impact on metabolism of humans and, in general, all living beings (*EFSA*, 2015).

A wide range of physiological uses of phosphorus is possible because it exists in various inorganic and organic forms in the human body. Phosphorus is mainly present as inorganic phosphates in serum and intracellular fluids, while bones contain apatite forms, and soft tissues and extracellular fluids contain organic phosphates (*EFSA*, 2015).

Excess phosphorus/phosphate intake can have adverse effects, such as hyperphosphatemia, hyperparathyroidism, skeletal deformations, bone loss and ectopic calcification (*Ritz et al.*, 2012). Considering all relevant and available scientific data regarding phosphorus dietary intake, EFSA set the adequate intake (AI) of phosphorus to 160 mg/day for infants aged 7–11 months, between 250 and 640 mg/day for children, and 550 mg/day for adults (*EFSA*, 2015).

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Along with naturally present forms of phosphorus, many processed foods contain several phosphate food additives. Phosphates are very important additives in the meat industry, because they improve water retention capacity, emulsification, oxidative and microbiological stability, softness and juiciness of meat products (*Pinton et al*., 2021). Current EU and national legislation on food additives (*EC 1333/2008*, 2008*; Sl. Glasnik RS 53/2018*, 2018), authorised the most relevant group of phosphate additives as "phosphoric acid — phosphates — di-, tri- and polyphosphates", and assigned E numbers 338–452. These additives are the most common source of inorganic phosphates, beside naturally present phosphates, in food. Legislation (*EC*, 1333/2008, 2008; *Sl. Glasnik RS 53/2018*, 2018) also establishes maximum permitted levels of phosphate additives in food. In general, levels of E338-452 additives in meat products are authorised up to 5000 mg per kg (0.5%). According to the legislation, these additives may be added individually or in combination, and their maximum level has to be expressed as P_2O_5.

Analytical determination of phosphates in meat products includes various physical and chemical techniques. Gravimetric, inductively coupled plasma (ICP) and spectrophotometric methods can only be used for direct determination of total phosphorus. Methods based on thin layer (TLC) and ion chromatography (IC), nuclear magnetic resonance (NMR) and electrophoresis have the ability to identify and quantify different inorganic phosphate ions, being more suitable for direct determination of phosphate additives in meat products (*EFSA*, 2019). On the other hand, methods for determination of total phosphorus were used for calculation and estimation of added phosphates in meat products in conjunction with determination of protein content (*ISO 937:1978*, 1978; *Dimitrovska et al.*, 2019). All mentioned techniques and methodologies have their advantages and flaws for the determination of the phosphate content in meat products. TLC, ion chromatography and capillary isotachophoresis measure phosphates directly, but may not be able to differentiate between added and naturally occurring phosphates. Other methods, such as NMR, thermo-differential-photometry and microwave dielectric spectroscopy, are not used for routine determination of added phosphates, and have the same limitation regarding phosphate origin (*Campden BRI Report*, 2012). Also, all reported instrumental techniques are unable to quantify polymerised phosphate higher than triphosphate. Of all available instrumental methods, IC is the most commonly used. However, IC cannot be successfully applied to the full range of foodstuffs permitted to contain phosphate additives (*EFSA*, 2019). For the simultaneous determination of phosphates and condensed phosphates using IC, systems employing gradient elution conditions with suppressed conductivity detection is needed (*Metrohm*, 2019). It is recommended that sample preparation times should be as short as possible and should include steps to deactivate phosphatase enzymes (*EFSA*, 2019).

The aim of this research was to point out the specific difficulties in interpreting results and discrepancies that occur during implementation of the current legislation regarding phosphate additives, based on results from applying the gradient ion chromatography method to determine the phosphate content of meat products.

2. Materials and methods

2.1. Chemicals

All standard chemicals and reagents were purchased from Merck KgaA, Darmstadt, Germany. Ultrapure water, ≥18 MΩ, were obtain by ELGA DV-25 and ELGA Ultrapure (LabWater, Lane End, High Wycombe, UK).

2.2. Samples

Meat products were part of regular controls of food quality and safety parameters, obtained from retail and from producers and importers. The research included 440 samples: 119 cooked sausages, 14 fresh sausages, 35 bacons, 117 fermented sausages, 90 dried and 65 smoked meat products.

2.2.1. Sample preparation

Samples for determination of total phosphorus were prepared according to the procedure described in the corresponding reference method (*ISO 13730:1996*, 1996). Preparation of samples for IC determination included extraction of phosphates from each homogenised sample with hot ultrapure water, centrifugation and filtration of the extract after cooling.

2.3. Methods

2.3.1. Total phosphorus content

Total phosphorus in the meat products was determined according to the spectrophotometric procedure described in the reference method (SRPS ISO 13730:1999, i.e. *ISO 13730:1996*, (1996)).

2.3.2. Ion chromatography

IC with conductometric detection was used for phosphate determination in the meat products. The IC system consisted of an 858 Professional Sample Processor, 930 Compact IC Flex with Oven/SeS/PP, and Conductivity Detector, all from Metrohm AG, Herisau, Switzerland. The separation column was Metrosep A Supp 7 250/4.0, also from Metrohm, and separation of anions was achieved by a mobile phase gradient in accordance with the original method provided by manufacturer (*Metrohm*, 2019).

2.4. Statistics

Preparation of data and statistical analysis were performed in MS Office 2016 Excel.

3. Results and discussion

The IC method, originally designed for determination of chlorates, thiocyanates, thiosulphates and perchlorates along with fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulphite and sulphate (*Metrohm*, 2019), was validated for determination of phosphate, diphosphate and triphosphate in meat products. Chromatograms of a meat product with declared added diphosphate and polyphosphate and the standard solution of phosphate, diphosphate and triphosphate, concentrations of 1 mg per litre each, are shown in Figure 1.

Several factors should be taken into consideration in interpretation of results. First of all, IC, as well as any other instrumental technique, cannot separate condensed phosphates higher than triphosphate (*EFSA*, 2019). However, according to the same source, polyphosphates (especially in aqueous solution such as the environment within meat products) are constantly degraded through their tri- and di- forms to phosphates, deeming measurement of individual ion species meaningless. This explains the legal requirement for the maximum permitted level of “phosphoric acid — phosphates — di- tri- and polyphosphates” to be expressed as P_2O_5, with the general remark that “The additives may be added individually or in combination”. In that context, it should be noted that referring to this (or any other) analytical

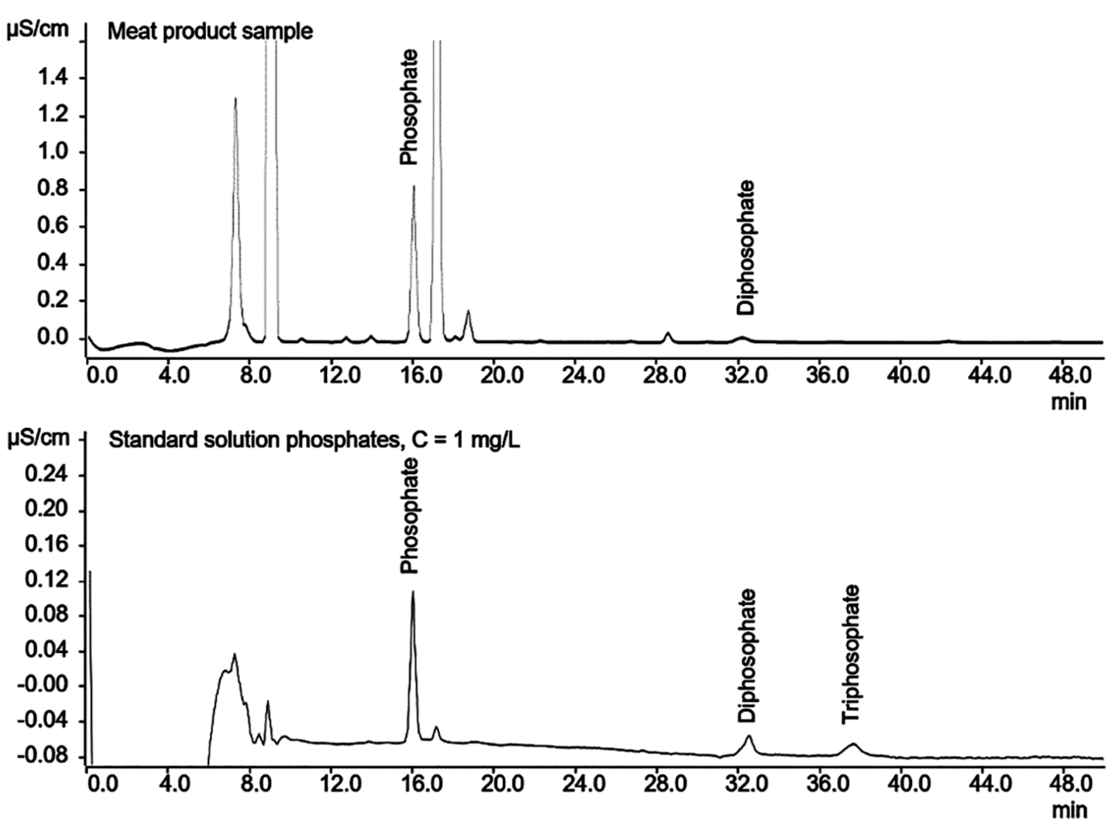

Figure 1. Chromatograms showing phosphate in a meat product and in the standard solution of mixed phosphate, diphosphate and triphosphate, each 1 mg per litre.

procedure for phosphate measurement as "determination of added phosphates", which can often be heard in both expert and lay circles, is misleading. Only total phosphate ions are measured. Their origin in the sample can be either from their natural presence in raw material (including, e.g., from water), or from utilization of phosphate-based food additives. However, the specific measurement of additive-originating phosphates (although desirable) is currently not required by the legislation, precisely for that reason. Maximum permitted levels are set at 5 g per kg for practically all groups of meat products taking into account both (relatively constant) natural presence and the technologically justified utilization of phosphate-based additives.

On the other hand, total phosphorus content, the parameter designed to monitor usage of phosphate-based additives, became less significant with the application of IC and was removed from the European Union legislation. In Serbia though, the national regulation on quality of meat products still sets the maximum permitted level of phosphorus at 8 g per kg (expressed as P_2O_5). This can explain the wrong perception of IC methods as tools for determination of "added" phosphates.

Results of determination of phosphates and total phosphorus content in meat products according to current legislation (*EC 1333/2008*, 2008; *Sl. Glasnik RS 53/2018,* 2018; *Sl. Glasnik RS 50/2019*, *34/2023*, 2023) are presented in Table 1.

As previously mentioned, usage of phosphates is authorised by EU and Serbian regulations on food additives (*EC, 1333/2008*, 2008; *Sl. Glasnik RS 53/2018*, 2018), and in Serbia, total phosphorus and protein contents were set by regulation on the quality of minced meat, meat semi products and meat products (*Sl. Glasnik RS 50/2019, 34/2023*, 2023). The maximum amount of total phosphorus in all meat products in Serbia was limited to 8 g per kg, expressed as P_2O_5.

Considering the number of non-compliant results for phosphates, the greatest number of such meat products were classified in two groups, fermented sausages and dried meat products. Current Serbian regulation (*Sl. Glasnik RS 50/2019, 34/2023*, 2023) does not require determination of total phosphorus in these categories, because these products have been dried in the process of manufacturing, and loss of moisture continues during the entire time they are stored. Consequently, the amounts of all other, non-water ingredients increase over time, including the phosphate content. Results of quality parameters determined in these products show that most of the non-compliant products were of good quality, but, according to Serbian and EU legal requirements (*EC, 1333/2008*, 2008; *Sl. Glasnik RS 53/2018*, 2018), the phosphate contents in the non-compliant products exceeded the maximum permitted level, regardless of the fact that di- and triphosphates were not detected.

4. Conclusion

Although IC is one of the most suitable instrumental methods for determining and quantifying inorganic phosphates in meat products, it has limitations that prevent its full application for the analysis of phosphate additives (*EFSA*, 2019). Unfortunately, other methods are also not able to fulfil the

Table 1. Phosphates and total phosphorus determined in meat products

Meat product	Total samples	Compliant		Non-compliant	
		Phosphates	Total Phosphorus	Phosphates	Total Phosphorus
Cooked sausages	119	114	115	5	4
Fresh sausages	14	14	14	0	0
Bacon	35	32	34	3	1
Fermented sausages	117	97	-	20	-
Dried meat products	90	68	-	22	-
Smoked meat	65	56	58	9	7
Total	440	381	221	59	12

requirements in this field (*EFSA*, 2019). Bearing in mind the method's limitations and advantages, it can be only said with certainty that IC can determine the total content of inorganic phosphates present in the product at the time of analysis.

From an analytical point of view, since almost every ingredient in a meat product contains some amount of inorganic phosphates (even the water used in production), it would be more suitable to speak of the total inorganic phosphates present in the product, rather than added phosphates.

In general, the category of non-heat-treated processed meats had a higher relative number of meat products that exceeded the maximum permitted level of 5000 mg of phosphate per kg compared to the cooked and fresh sausages (4.2% and 0%, respectively). This was despite the fact that phosphate additives are used less frequently in the production of non-heat-treated processed meats than in the cooked and fresh sausages. Considering that phosphate content naturally increases with drying and storage time, and these products are consumed less often than cooked sausages, it would be advisable to revise the Serbian legislation regarding the maximum permitted levels of phosphate additives in processed meats.

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