Monitoring of polyphosphate levels in animal source products collected in Italy by means of ion chromatography with suppressed conductivity detection

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Abstract

Polyphosphates (PPs) constitute a class of food additives widely used due to their ability to exercise different useful activities. The food safety concern about the use of PPs in food is both the possible non-declared addition and some health effects, such as bile duct stones, decrease in oligo-element absorption, and allergic reactions in susceptible people. In this study, an analytical method based on ion chromatography with conductivity detection was applied for the detection and quantification of PPs in 238 samples of animal-derived products such as meat, dairy, and fish products. A contribution to risk assessment was also included. The monitoring confirmed the absence of non-compliant results. All concentrations of PPs were indeed lower than the legal limits set in European Regulation No. 1333/2008. Moreover, no residue of PPs was detected (> limit of quantification: 0.09 g kg^{-1}) in samples where it was not reported on the product label. No PPs were detected in mollusks, meat-based preparations, semi-ripened, unripened, and spun paste cheese, while they are widely used in surimi, with concentrations in the range of 0.1-0.5 g kg^{-1}. The highest concentrations were quantified in a würstel sample (4.7±0.3 g kg^{-1}) and a spreadable cheese sample (8.9±0.7 g kg^{-1}). Considering that the high exposure scenario together with a very susceptible population group (toddlers) were taken into account for this risk exposure study and that the highest admissible daily intake obtained was equal to 10.4%, the assessment demonstrated that the actual use of PPs in food does not pose a risk for food safety.

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Introduction

Polyphosphates (PPs) constitute a class of food additives used as stabilizers in several foodstuffs. Chemically, they are composed of orthophosphate units linked by phospho-anhydride bonds to form ramified and linear chains from two (diphosphate or pyrophosphate) to several hundred units (PPs) (Jolley and Purslow, 1988).

High amounts of PPs are used in food due to their ability to exercise different useful activities. For instance, their addition to meat products allows for a decrease in the amount of sodium chloride added to the product, reducing the health risk from sodium overconsumption (Jolley and Purslow, 1988), an increase in the retained water after cooking (Trout and Schmidt, 1983; Offer and Knight, 1988; Li *et al*., 2017), and an increase in the extraction of myosin (Jolley and Savage, 1985; Shen *et al*., 2016). PPs are also used for the treatment of frozen and deep-frozen fish fillets, mollusks, and crustaceans, canned crustacean products, surimi and similar products, fish and crustacean paste, processed frozen and deep-frozen mollusks and crustaceans, where their addition is permitted by European Regulation 1333/2008. Other examples of food types where the addition of PPs is permitted in Europe are creams, fat, cheese products, fruit and vegetable preparations, confectionery, flours, breakfast cereals, *etc*., with limits of addition varying from 800 to 30000 mg kg^{-1} (European Parliament and Council, 2008; European Commission 2011). The food safety con-

cerns about high intakes of PPs from the diet are the possible pathogenesis of bile duct stones, the significant decrease in the absorption of some oligoelements such as calcium, and some allergic reactions in susceptible people (Carey, 1992; EFSA Panel on Food Additives and Flavorings *et al.*, 2019). However, another important food safety aspect is the not-permitted addition of PPs in unprocessed products which is considered a food sophistication, so the European Commission has recently published a call for data on the permitted food additives [phosphoric acid (phosphates), di-, tri- and polyphosphates] also to evaluate the extent to which these additives are used without declaration (European Commission, 2023). There are some approved and standardized reference methods for the detection of added PPs in foods. The ISO 5553:1980 official method is based on thin layer chromatography and applicable to meat sample analysis (ISO, 1980). The indirect determination of PPs can be accomplished by using ISO/TS/18083:2013 and UNI 10591:1997 for the analysis of cheese and meat products, respectively (Ente Nazionale Italiano di Unificazione, 1997; ISO, 2013). These 2 methods are based on the photometric detection of total phosphorous present in the sample. However, some authors have called into question the reliability of these methods (Sekiguchi *et al.*, 2000; Iammarino *et al*., 2014) since other food additives or ingredients containing high ratios of phosphorus can compromise method selectivity, also causing "false positive" responses. Thus, the lack of a well-established and reliable analytical method is the main reason why no survey on levels of PPs in food is still available (European Commission Joint Research Center - Institute for Reference Materials and Measurements, 2013). In order to improve the analytical tools for this type of determination, Iammarino and Di Taranto (2012) developed and validated a procedure based on ion chromatography with conductivity detection that is applicable to the most important animal-derived products, such as meats, cheese, and seafood. This method was also compared to indirect photometry, as described in the standard procedures cited above, resulting in higher accuracy and a lower percentage of "false positive" responses (Iammarino *et al.*, 2020; Vita *et al.*, 2022). In this study, this analytical method was applied for comprehensive monitoring focused on evaluating the residual levels of PPs in 238 samples of animal-derived products such as meat, dairy, and fish products. A contribution to risk assessment has also been given.

Materials and Methods

Chemicals

Sodium hexametaphosphate (65-70% P_2O_5 basis) was supplied by Merck KGaA (Darmstadt, Germany), and carbonate-free sodium hydroxide (50%, w/w) was purchased from Fisher Scientific Italia (Rodano, Milan, Italy). All solutions were prepared by using ultrapure water with a specific resistance of 18.2 MΩ-cm, produced by a Milli-Q RG unit, Millipore (Bedford, MA, USA). The solutions used as mobile phase, composed of different concentrations of sodium hydroxide, were filtered using 0.45-µm membranes and degassed with nitrogen. The standard solutions of PPs were prepared from the 1000 mg L^{-1} stock solution, diluting with ultrapure water to obtain the following concentrations: 12.5, 25, 50, 100 and 200 mg L^{-1}.

Apparatus

The chromatographic determinations were carried out by using a high-pressure ion chromatography system (Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System, Thermo Fisher Scientific Inc., Waltham, MA, USA) composed of a gradient mixer (Dionex GM-4, 2 mm), an injection valve with a 25-µL loop, a SP Single Pump (ICS-6000), a Dionex anion self-regenerating suppressor (ADRS 600, 4 mm) set at the recommended voltage, and an electrochemical detector set to conductivity mode. The column compartment temperature was set at 20°C and the chromatographic column was the IonPac AS11 (250×2 mm i.d., particle size: 9µm) (Thermo Fisher, Scientific Inc., Waltham, MA, USA). The separation of PPs was obtained by using the following gradient elution, based on 2 solutions, 10 mM NaOH (A) and 80 mM NaOH (B): isocratic step at 100% A for 4 minutes, from 0% to 15% B in 1 minute, isocratic for 2 minutes, from 15% to 50% B in 1 minute, isocratic for 9 minutes, from 50% to 0% B in 1 minute and a final re-equilibration step at 100% A for 2 minutes (total run time: 20 minutes). The system was interfaced to a personal computer *via* proprietary network chromatography data system (Chromeleon 7.2.8, Thermo Fisher Scientific Inc., Waltham, MA, USA) for data acquisition/processing and instrumentation control.

Sample preparation for ion chromatography analysis

Regarding sample preparation, the samples were collected from markets located in 2 Italian regions, Apulia and Basilicata, and then stored in laboratory at -18°C until analysis. After sample homogenization (~40-60 g), a 4 g portion of homogenized matrix was placed in a 50 mL Falcon® tube (Merck KGaA, Darmstadt, Germany) together with 40 mL of ultrapure water, then vortexed for 1 minute. After centrifugation at 250× g for 5 minutes at room temperature, the supernatant (at least 2 mL) was filtered using a 0.2 µm Minisart® NML surfactant-free cellulose acetate syringe filter (Sartorius AG, Goettingen, Germany) and then injected in the high pressure ion chromatography (HPIC) system with no further purification step. The samples were analyzed in duplicate and the results were expressed as the mean of 2 measurements. It is well-known that PPs easily degrade to o-phosphate components.

Thus, all samples and standard solutions were analyzed by ion chromatography within 2 hours from preparation, storing at 3±1°C until HPIC injection. According to Regulation 1129/2011, the final concentration was expressed as P_2O_5, then converted by multiplying the final amount by 0.675, a factor that takes into account both the PPs/P_2O_5 molar ratio and the respective molecular weights (European Commission, 2011). An example of a chromatogram obtained from the injection of a standard solution of PPs is shown in Figure 1.

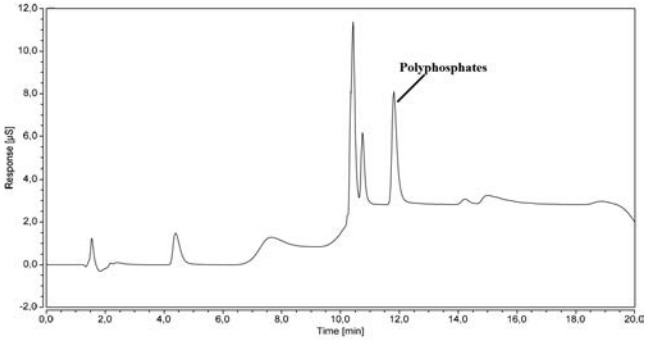

Figure 1. Chromatogram of polyphosphates standard solution at a concentration of 200 mg L^{-1}.

Method validation

The analytical procedure was validated following an in-house validation model, according to the relevant legislation and the most representative guidelines (Youden and Steiner, 1975; Miller and Miller, 1993; Hund *et al.*, 2001; European Commission, 2002; Thompson *et al.*, 2002; EURACHEM/CITAC, 2012; European Parliament and Council, 2017; Iammarino, 2019). The most important validation parameters that characterize the analytical method are summarized in Table 1 (Iammarino and Di Taranto, 2012; Iammarino *et al.*, 2013).

Risk exposure study

Given the lack of analytical data about the levels of PPs in food, no risk exposure study has been published so far, comprising any kind of possible contribution to the estimation. Thus, the data obtained from this monitoring have been used to carry out a risk exposure study. The exposure estimates are usually elaborated using the minimum, mean, and maximum levels detected for target compounds in each food type monitored.

These amounts are then combined with relevant food consumption data in order to evaluate 3 different exposure scenarios (low, average, and high) which represent the likely exposure across the population (Lee, 2018). Taking into account that the concentrations of PPs detected during this monitoring were well below the legal limits, only the high exposure scenario was elaborated.

Moreover, the assessment was provided only for a very susceptible category, toddlers, considering 12 kg as body weight (EFSA Scientific Committee, 2012). The admissible daily intake (ADI) used for exposure studies is 40 mg/kg body weight (bw) per day, expressed as phosphorus (EFSA Panel on Food Additives and Flavourings *et al.*, 2019). Thus, the P_2O_5 values were converted to phosphorous, considering a factor of conversion of 0.44, which takes into account both the molar ratio and the respective molecular weights. Regarding food consumption, reference data were obtained from the INRAN-SCAI 2005-06 Italian survey provided to the European Food Safety Authority (Leclercq *et al.*, 2009) particularly the appendages 6-B5 (meat, sausages, and meat substitutes), 7-B2 (fish and seafood) and 8-B3 (milk and derived products and milk substitutes).

Results and Discussion

The results obtained by analyzing 238 samples of meat, dairy, and fish products are reported in Table 2, while in Figures 2 and 3, some chromatogram examples related to samples containing and not containing PPs are shown.

The first important comment, from a food safety point of view, is the absence of non-compliant results. Indeed, considering the legal limits set in Regulation 1333/2008, the concentrations of PPs detected in all samples were lower. Moreover, no residue of PPs was detected [> limit of quantification (LOQ): 0.09 g kg^{-1}] in any samples where PPs were not reported on the product label, confirming that the additives were only quantified in samples where they were regularly declared. From Table 2, another significant result is appreciable for some food types, such as mollusks, meat-based preparations, semi-ripened, unripened and spun paste

Table 1. Analytical method validation parameters.

Analyte	LOD (g kg^{-1})	LOQ (g kg^{-1})	Mean recovery (n=24)*	RSD_r % (n = 24)*	Measurement uncertainty %	Accreditation	Robustness and selectivity
Polyphosphates	0.03	0.09	93.5%	2.7	7.5	Yes	Seafood Meat Cheese

LOD, limit of detection; LOQ, limit of quantification; RSD_r, relative standard deviation; *spiking tests at 4 fortification levels, 6 repetitions each.

Table 2. Results obtained by analyzing 238 samples of animal-derived products.

Food type	N° of analysed samples	N°>LOQ*	% >LOQ	Concentration detected (g kg^{-1})	Related legal limit (g kg^{-1})*
Unprocessed fish	57	1	1.7	0.3	5
Processed fish (Surimi)	7	4	57.1	0.1-0.5-0.3-0.5	1
Crustaceans	6	1	16.7	0.1	5
Molluscs	16	0	0	-	5
Fish-based preparations	10	1	10.0	1.8	5
Meat-based preparations	15	0	0	-	5**
Meat products	25	1	4.0	4.7	5
Semi-ripened cheese	33	0	0	-	2
Melted cheese	20	4	0	1.5-0.3-0.4-0.1	20
Unripen cheese	17	0	0	-	2
Spreadable cheese	13	3	23.1	8.9-0.2-0.3	20
Spun paste cheese	19	0	0	-	2
Total	238	15	6.3	0.3-0.1-0.5-0.3 0.5-0.1-1.8 4.7-1.5-0.3-0.4 0.1-8.9-0.2-0.3	-

N, number; LOQ, limit of quantification; *as defined in the Regulation 1333/2008; **only meat preparations specified in the restrictions/exceptions of Regulation 1333/2008/EC.

cheese, where PPs are substantially not used as food additives. On the contrary, the greater use of PPs (57% of samples with concentration >LOQ) was verified in surimi samples, with concentrations in the range 0.1-0.5 g kg^{-1}.

The highest concentrations were quantified in 2 samples, a würstel and a spreadable cheese, equal to 4.7±0.3 and 8.9±0.7 g kg^{-1}, respectively. Both concentrations were lower than the respective legal limit, 5 and 20 g kg^{-1}, defined in the relevant European Regulation for heat-treated meat products and processed cheese, respectively (Table 2).

The monitoring also confirmed the possibility of obtaining false-negative responses when ion chromatography is used to analyze seafood (Iammarino *et al.*, 2020). Indeed, a residue of PPs higher than the method LOQ was not detected in 2 surimi samples out of 6 analyzed (where PPs were declared on the label), corresponding to 33.3%.

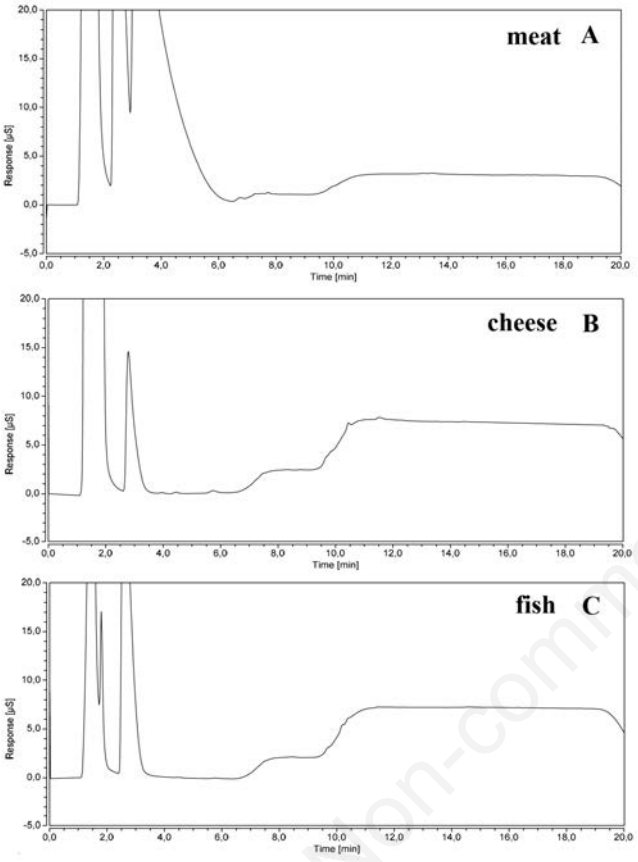

Figure 2. Chromatogram examples. Cooked ham (A), processed cheese (B) and unprocessed fish (C) samples with no detected polyphosphates.

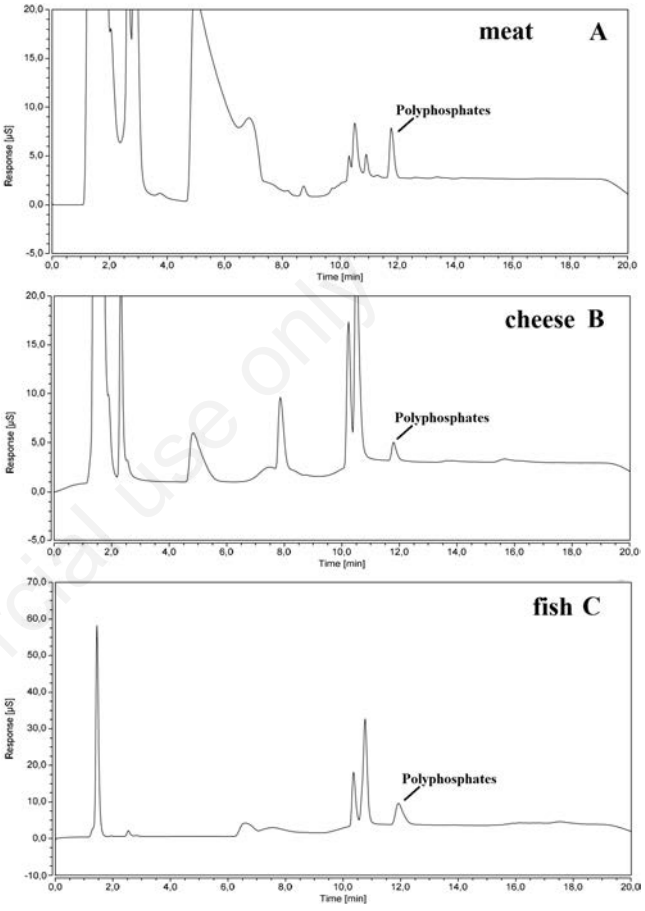

Figure 3. Chromatogram examples. A) Würstel sample (diluted extract) with polyphosphates detected at a concentration of 4.7 g kg^{-1} (as P_2O_5); B) cheese slices sample with polyphosphates detected at a concentration of 1.5 g kg^{-1} (as P_2O_5); C) crab claws sample with polyphosphates detected at a concentration of 1.8 g kg^{-1} (as P_2O_5).

Table 3. Risk exposure for toddlers under high exposure scenario.

Food type	Toddlers mean daily consumption (g)*	Polyphosphates daily intake (mg of P_2O_5)	Polyphosphates daily intake (mg of P)	% ADI**
Unprocessed fish (cod)	40	12.0	5.0	1.0
Processed fish (surimi)	3	1.5	0.6	0.1
Crustaceans (shrimp)	40	4.0	1.8	0.4
Fish-based preparations (crab claws)	63	113.4	49.9	10.4
Würstel	21	98.7	43.4	9.0
Melted cheese	1	1.5	0.7	0.1
Spreadable cheese	1	8.9	3.9	0.8

ADI, admissible daily intake; *INRAN-SCAI 2005-06 - B2 (fish), B3 (cheese), B5 (meats); **% ADI calculated using 12 kg as reference body weight (EFSA Scientific Committee, 2012).

This missing detection can be due to both the low amount of PPs added and the prolonged storage before analysis, which leads to the additive degradation of o-phosphate units. Taking into consideration the high legal limit set in the European Regulation for this type of sample (1.0 g kg^{-1}), and the concentrations lower than the method LOQ (0.09 g kg^{-1}), no food safety concern can be considered underestimated in the case of missed detection of PPs. Other than these overall considerations, a contribution to risk assessment was also made. The results obtained from the risk exposure study are reported in Table 3 (EFSA Scientific Committee, 2012).

The ADI percentages calculated for unprocessed and processed fish, crustaceans, and melted and spreadable cheese were all ≤1.0, in the range 0.1-1.0%, demonstrating that the phosphorous intake due to PPs use in such products is substantially low. Higher percentages were calculated for fish-based preparations and würstel, equal to 10.4% and 9.0%, respectively. Taking into account that the high-exposure scenario together with a very susceptible population group (toddlers) were considered for the risk exposure study and that the highest ADI percentage obtained was equal to 10.4%, the assessment demonstrated that the actual use of PPs in food does not pose a risk for food safety.

Taking into account the overall results obtained from this monitoring, it is possible to confirm that the topic of PPs in food needs to be properly addressed. Indeed, it is possible to define 2 levels of food safety concern: one direct and one indirect. The first one, direct, is related to the health effects of PPs and seems not particularly significant since the amounts of additives usually added to food are substantially low and the toxicity of these food additives is not high. The second one, indirect, needs deepening. The indirect concern is related to the not-declared addition of PPs used to mask the low quality of raw materials (*i.e.*, cooked ham) and/or extend products shelf-life (*i.e.*, fresh fish). The indirect concern is clearly evident, especially in the last case, where the treatment of fish with PPs masks deterioration by retaining more water, even after different days of storage. Thus, the possible microbial growth (also pathogens, if present) and increase in concentration of harmful substances (*i.e.*, histamine) represent important matters of concern in food safety.

These final considerations have relevant repercussions from an analytical point of view. Indeed, considering the very high legal limits set at European level, the amounts of PPs added into food (not so high), and the degradation of PPs to o-phosphate, diphosphate, and triphosphate, which is very variable depending on many parameters, quantitative analysis seems not particularly significant (indeed, ion chromatography does not allow the quantification of the original amount of PPs added into the product but the actual residue present on consumption).

On the contrary, taking into consideration the comments related to the "indirect" concern, the analytical method used during official food controls must be able to qualitatively ascertain the addition of PPs to food.

This means that a possible standardization of a novel analytical method for this type of determination should opt for qualitative determination in order to ascertain the treatment of food with PPs where it is not declared.

Conclusions

In this study, 238 samples of animal-derived products were analyzed by means of ion chromatography with conductivity detection for the determination of PPs.

No non-compliant result was obtained, with concentrations of PPs always in compliance with European legal limits, and no not-declared addition was registered.

PPs are widely used in surimi, with concentrations in the range 0.1-0.5 g kg^{-1}, and the highest concentration was quantified in a spreadable cheese sample (8.9±0.7 g kg^{-1}).

The risk exposure study allowed us to conclude that the actual use of PPs in food does not pose a risk to food safety. The focus on such a topic should be moved more to the not-declared addition of PPs in food, which could mask low-quality products, also from a food safety point of view.

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