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PHOSPHORUS CONTENT, NATIVE AND ADDED TO PIKE-PERCH (*Sander lucioperca*) FILLETS, SOLD ON THE EUROPEAN MARKET AND ITS EFFECTS ON TOTAL PRODUCTS' QUALITY

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Abstract

The paper presents the phosphorus content of pike-perch (Sander lucioperca) reared in Germany, in natural ponds and water recirculating systems respectively, compared with the same element measured from fillets imported from Kazakhstan and Russia and marketed in EU retail units from Hamburg Hanseatic City. It is based on the research data obtained in Max Rubner Institute, the Department of Safety and Quality of Milk and Fish Products among federal research institutes within the remit of the German Federal Ministry of Food and Agriculture (BMEL). The data have been processed into the proximate analysis parameters: pH, TVB-N, humidity and dry matter, ash percent, total phosphate, fat, protein and salt content. During the 10 months period of study (October 2013 - August 2014), were analyzed different sample types of pike-perch, refrigerated inland whole fish and frozen imported fillets. The phosphates content of fresh German pike-perch was situated approximately between 4.36 - 4.56 g P_2O_5/ kg of fish muscle, whereas the same parameter in frozen imported fish fillets analyzed were between 2.63 - 3.59 g P_2O_5/ kg. In conclusion, the use of added phosphates is usually suitable for fish fillet production. However, due to their water-binding capacity which also could determine improved juiciness of pike-perch fillets, the added phosphates negatively influenced the fillets' quality by forming an important amount of glaze, which after thawing led to unwanted weight loss, without bringing any threats to fish safety or consumers' health. The water loss during thawing caused a significant depletion of previously added phosphates.

***Key words**: pike-perch, native phosphates, added phosphates, marketed frozen fillets, unprocessed pike-perch.*

INTRODUCTION

Fish is an important food both for humans and animals. Fish farming is an important branch of aquaculture in many EU and non-EU countries.
Protein - rich foods, such as seafood and meat products contain phosphorous compounds such as nucleotides, phospholipids, together with naturally occurring orthophosphates. The large range of natural orthophosphate contents (0.11-4.8% - 0.026-1.1% in terms of phosphorous content), makes it hard to detect added phosphates by quantitative analysis alone (Lawrie, 1998; Lee et al., 1998; Ünal et al., 2004).
The addition of phosphates is allowed in frozen products but not in salted or fresh products. The maximum quantity of added phosphates allowed in frozen products is 5 g P_2O_5/kg of the product (phosphorus pentoxide $P_2O_5 \approx 2.29 \times P$; orthophosphate $PO_4^{3-} \approx 3.06 \times P$). Of the list of additives authorized, only a few are a source of phosphorus and they are displayed on labels with a letter and number format: phosphoric acid (E338), phosphates (E339, E340, E341, E343), diphosphates (E450), triphosphates (E451) and polyphosphates (E452) (EU, 2011; Lou-Arnal et al., 2014).
Polyphosphates have been used by some produces in the production of salted fish in the Nordic countries (Lindkvist et al., 2008) in the belief that they are processing aid. The use depends on the export country, product but also on producer / customer.
High levels of phosphorus are related to the development of arteriosclerosis and bone disease in patients with chronic kidney disease (CKD) (Tentori et al., 2008). Phosphorus intake is also a public health problem given its impact on cardiovascular risk in the general population

("new cholesterol") (Dhingra et al., 2007; Sax, 2001; Calvo & Uribarri, 2013).
Hyperphosphatemia is a common disorder in patients with CKD, and may result in hyperparathyroidism and renal osteodystrophy. Hyperphosphatemia also may contribute to deterioration vascular calcification and increase mortality. Hence, correction and prevention of hyperphosphatemia is a main component of the management of CKD. This goal is usually approached both by administering phosphorus binders and by restricting dietary phosphorus (P) intake. Dietary intake of phosphorus (P) is derived largely from foods with high protein content or food additives and is an important determinant of P balance in patient with CKD. Food additives (PO_4) can dramatically increase the amount of P consumed in the daily diet, especially because P is more readily absorbed in its inorganic form. In addition, information about the P content and type in prepared foods is often unavailable or misleading. Therefore, during dietary counselling of patients with CKD, we recommended that they consider both the absolute dietary P content and the P-to-protein ratio of foods and meals including food additives (Kido et al., 2012).
Intake of natural foods that are not pre-cooked and the phosphorus content of some soft drinks (particularly cola) are important aspects that we should consider (Kalantar-Zadeh, 2010).
Currently, the maximum amount allowed in Switzerland and the EU is 5 g P_2O_5/kg for frozen fishery products (Manthey-Karl et al., 2014). This value does not include the natural phosphorus content which is on average 2.2 g/kg (≙5.7 g P_2O_5/kg) in the range of 1.0 to 4.0 mg P/kg. This variability may be related to biological factors and may also be caused by bone fragments (Wheeler & Hebard, 1981).
In this context, the paper presents a comparative analysis of the content of phosphorus pentoxide P_2O_5 in the fresh fish and frozen *Sander lucioperca* fillets produced in Germany, Kazakhstan and Russia, in order to highlight the influence of added phosphates, on the total products' quality.

MATERIALS AND METHODS

Quantification of total phosphate content is usually carried out by spectroscopic analysis. The sampling preparation is based on a decomposition of polyphosphates to orthophosphate in the presence of sulfuric or trichloroacetic acid (Jastrzębska et al., 2008) (Figure 1).

Figure 1. Ashed sample preparation for phosphates extraction

The orthophosphates react with ammonium molybdate and ammonium vanadate in nitric acid (HNO_3) and a yellow precipitate is formed. The concentration of phosphovanadomolybdate is used to calculate the content of phosphate or phosphorus.
In this study, the total phosphorus content was measured by the spectrophotometric reference method, adapted to the German official rules (§ 64 LFGB 06.00-9). The homogenized fish sample was dried and calcined, then the ash was hydrolyzed with nitric acid and filtered (Figure 2).

Figure 2. The ash was dissolved in 10 ml of dilute nitric acid. The filtrate was transferred quantitatively to a 100 ml volumetric flask and made up to the mark with distilled water

Briefly summarized, the ash from homogenized fish samples (5 g) were dissolved in 20% HNO_3 (v/v) by heating in a boiling water bath for 30min. After addition of 0.25% aqueous ammonium monovanadate (w/v) and 5% ammonium heptamolybdate (w/v) to an aliquot of the nitric acid solution (~ 20%), the mixture formed a yellow-colored complex, whose extinction was photometrically measured at 430 nm, using a Varian Cary 50 UV-Vis Spectrophotometer Agilent (Figure 3).

Figure 3. Measurement of the extinction 15 minutes after mixing the reagents (λ = 430 nm)

Extinction measured was directly proportional to phosphorus concentration.
By using this method were analyzed 25 fillet samples of *Sander lucioperca* imported from Kazakhstan and Russia and 10 samples of raw refrigerated *Sander lucioperca* from Germany, respectively.
Quality assurance of the chemical analysis was performed by analyzing a reference material (muva-Referenzmaterial Nahrungsergänzungsmittel 752; http://www.muva.de/). Results showed an excellent agreement with the certified value (Boiţeanu et al., 2014).
The data, obtained during the research, have been statistically processed and expressed calculating the average and the standard deviation. We carried out a phosphorus content repeatability study in fish, with five repetitions for each sample (Figure 4).

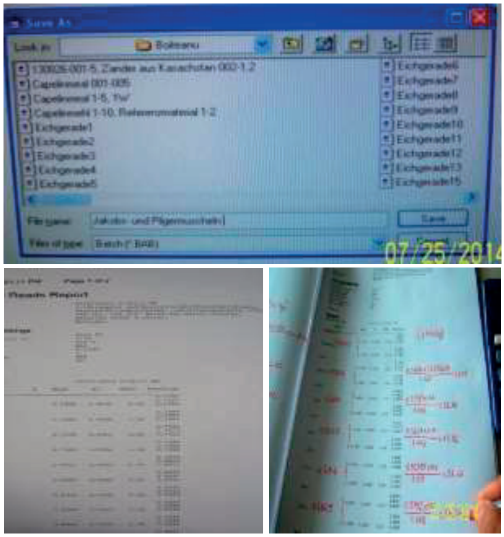

Figure 4. Results' reads and calculations

RESULTS AND DISCUSSIONS

Water is the most abundant component in its muscle, considering weight as well as volume (70-80%). As a main component, it influences the seafood sensory attributes, its shelf life and quality. However, a part of this water is lost during transportation, from its capture to its processing and posterior commercialization, through drip, evaporation and/or cooking (Toldrá, 2003; Gonçalves, 2004a; Gonçalves, 2004b).
The seafood processing companies have a great concern in retaining this water, first for economic reasons (seafood is sold by weight) and secondly, for the quality of the final product (Toldrá, 2003). On the other hand, an excessive loss of water can generate a great dissatisfaction on the part of the consumers for the following reasons: (a) the drip of the fish generates an undesirable appearance; (b) cooking reduces the size of the fish; (c) and mainly, the loss of the sensory attributes (juice, texture and colour) make the seafood less attractive (Gonçalves & Ribeiro, 2008).
The addition of polyphosphates improves the water retention during processing and may lead to an unjustified water uptake and increase in weight. Various phosphates have been widely accepted as additives in frozen fish and seafood (Manthey-Karl, 2015).

The phosphates soluble in the muscle liquid diffuse out of the muscle as the muscle is dehydrated at high salt contents (Þórarinsdóttir et al., 2010).
Polyphosphate solutions act inside the muscle fibres, causing the pH to increase, which ensures a more efficient absorption of water by the muscles, while reducing the loss of exudate during storage and improving the various properties of meat (Lemos et al., 1999).
By adding phosphates to raw muscle there is not only an increase in water retention capacity, but water remains in the muscles even after thawing and heat treatment. The condensed types of phosphates work effectively in this regard, as they promote protein dissociation and water retention. The addition of phosphates is strictly regulated and supervised in fish, crustaceans and molluscs, requiring, in any case, appropriate labelling. Addition of phosphates should perhaps only be carried out by brining. The presence of phosphate in brine used for injection does not improve the weight yields compared to use of salt only. Therefore, the main benefit is related with color and appearance of the fillets, i.e., the retarding effects of phosphate on oxidation and the maintenance of the natural color of the fish muscle. Brining could be an effective way to improve these parameters and at the same time led to lower increases in phosphate content (Þórarinsdóttir et al., 2010).
The Kidney Disease Outcomes Quality Initiative guidelines recommend a dietary ratio of 10-12 mg P/g protein (Lou-Arnal et al., 2013). Fish products represent serious sources of phosphorus intake. The regulatory framework does not bring any help in the effort of reducing phosphorus additives, since it considers them safe for public consumption and public health. There are categories of consumers (e.g., patients with CKD) that should carefully monitor their phosphorus intake.
Overcoming current obstacles and successfully decreasing the phosphorus intake suppose a collaborative effort to demonstrate that these additives possess harmful effects not only to CKD patients but also on the rest of the general population and they should be more strictly regulated.

Taking into account the imposed legal limits of P in fish and fishery products, the values of phosphorus pentoxide (g/kg) has been determined and recorded a decreasing trend in frozen fillets in comparison with refrigerated *S. lucioperca* (Table 1).

Table 1. Phosphorus pentoxide P_2O_5 in filleted pike-perch (*Sander lucioperca*), fish feed, and fish homogenate

Samples' type	P_2O_5 (g/kg) ± SD (n = 5)	Glaze content %
German pike-perch reared in natural ponds (Figure 7)	**4.4 ± 0.3**	-
German pike-perch reared in water recirculation systems	**4.6 ± 0.2**	**-**
Pike-perch fillets from inland waters of Kazakhstan (Figure 8)	**3.2 ± 0.6**	**11.6 ± 4.3**
	3.5 ± 0.3	**8.8 ± 2**
	3.6 ± 0.3	**12.9 ± 1**
Pike-perch fillets from Volga River, Russia (Figure 8)	**2.6 ± 0.4**	**11.7 ± 2**
Fish feed A	**21.7**	**-**
Fish feed B	**26.1**	**-**
Fish homogenate A	**18 ± 5**	**-**
Fish homogenate B	**19 ± 3.3**	**-**

This positive aspect was also determined by the reduced quantity of phosphorus pentoxide found in both imported and EU indigenous pike-perch, that did not exceed the limit of 5 g/kg P_2O_5. The fish farmers from Germany have selected the best diet based on the phosphorus content of reared fish in order to register a lower yield of P in the final products' composition.
In the North Eastern part of Germany, the average phosphorus yield in pike-perch homogenate from fish reared in water recirculation systems was 18.5 ± 3 g/kg P_2O_5 (Figure 5).

Figure 5. Pike-perch obtained from aquaculture in water recirculation systems

The fish was homogenized whole with head, skin, bones and intestines (Figure 6).

Figure 6. Whole homogenized pike-perch

Figure 7. Pike-perch obtained from aquaculture in German natural ponds

Figure 8. Frozen pike-perch fillets imported from Russia and Kazakhstan

The phosphorus pentoxide content was higher in German fish muscle compared with imported pike-perch fillet samples from Russia and Kazakhstan (Figure 9). The glaze content in pike-perch fillets exceeded 10% in most of analyzed samples (Table 1).

Adequate glazing (6-10%) of fish fillets prior to frozen storage protects the final product from dehydration, oxidation and quality loss. Excessive glazing (>12%) on the other hand may significantly affect the economic value and end user satisfaction of frozen fish fillets (Vanhaecke et al., 2010).

Our results shown an excess of glaze in both frozen fillets (Table 1) from Russia (with an average of 11.7 ± 2%) and Kazakhstan (with values between 8.8 ± 2% and 12.9 ± 1%) which led to a significant depreciation of the total products' quality.

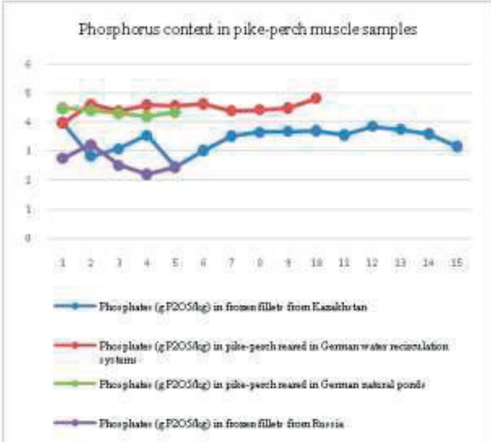

Figure 9. Fluctuation of phosphorus pentoxide (g/kg) in Pike-perch (*Sander lucioperca*) samples reared in four different sweet water sources

CONCLUSIONS

Fish products represent serious sources of phosphorus intake. Phosphorus based additives possess harmful effects not only to CKD patients but also on the rest of the general population and they should be more strictly regulated.

The regulatory framework does not bring any help in the effort of reducing phosphorus additives, since it considers them safe for public consumption and public health. There are categories of consumers (e.g., patients with CKD) that should carefully monitor their phosphorus intake.

The benefit of their use is a better water-binding and retention capacity which improves the sensory attributes of fish fillets.

In this study the observed side effect of phosphates consisted only in excessive weight gain of fish fillets with a medium value of glaze content situated in the range of 8.8 to 12.9%.

All samples analyzed here did nor overpass the legal limit imposed by the EU legislation (5 g added P_2O_4 /kg fish muscle). This value does not include the natural phosphorus content which is on average 5.7 g P_2O_5/kg.

The natural phosphorus content found in pike-perch samples from fish reared in two types of aquaculture systems in the North Eastern part of Germany was situated between normal limits (4.4-4.6 g P_2O_5/kg) and lower than the previously reported average.

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