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Influence of deep-fat frying process on phospholipid molecular species composition of *Sardina pilchardus* fillet

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ABSTRACT

The effects of deep-fat frying performed using different culinary fats (extra virgin olive oil, conventional sunflower oil and high-oleic sunflower oil) and different frying temperatures (160 and 180 °C) on the composition of the preponderant fish phospholipid classes, phosphatidylethanolamine (PE) and phosphatidylcholine (PC), were investigated on *Sardina pilchardus*. The total fish lipid fraction was injected into the HPLC system coupled on line with a second order mass spectrometer (MS–MS) by means of electronebulization interface (ESI), without a prior clean-up of the phospholipid fraction.

The deep-fat frying process caused significant changes on PE and PC molecular species composition of the fish fillet.

In all cases, the deep-fat frying process caused a significant ($P < 0.05$) increase in the relative proportions of the PE and PC species constituted by the combination of palmitic and docosahexaenoic acids. At the same time, a depletion of the percentage of the PE and PC species formed by two docosahexaenoic acid residues in fried fillets was registered. Anyway, this depletion was statistically significant for PC, but not for PE.

Both PE and PC compositions were not influenced by the frying temperature, whereas the nature of the culinary fat had an effect on the PC composition. Particularly, the frying tests with conventional sunflower oil produced a statistically significant increase of PC species containing saturated/polyunsaturated fatty acids and a significant decrease of PC species formed by the combination of two polyunsaturated fatty acids.

1. Introduction

Fish is an excellent source of nutrients such as essential amino acids, bioactive fatty acids, minerals, vitamins, chitin and antioxidants (Gebauer, Psota, Harris, & Kris-Etherton, 2006). Extensive epidemiological studies have demonstrated the protective role of fish and fish oil consumption against a wide range of diseases that are becoming more widespread in Western populations, including coronary heart diseases (Barringer & Harris, 2012; Calder & Yaqoob, 2009; Saravanan, Davinson, Schmidt, & Calder, 2010), type 2 diabetes (Malekshahi et al., 2012), inflammatory disorders (Simopoulos, 2008), cancer (MacLean, Newberry, Mojica, & Khanna, 2006) and neurodegenerative diseases (Freeman, Hibbeln, Wisner, & Davis, 2006; Huang, 2010).

The potential health and nutritional benefits of fish consumption are mainly attributed to the lipid fraction, which is primarily composed of phospholipids (PLs) and triacylglycerols (TAG) exceptionally rich in ω3 polyunsaturated fatty acids (ω3 PUFA). The strong and positive biological activities of ω3 PUFA have been confirmed by extensive research over the past several decades and various governments and health organizations are currently recommending dietary intakes for total ω3 PUFA of 1.4–2.5 g/day (American Heart Association Nutrition Committee, 2006; Harris et al., 2009). Nevertheless, in many societies of the Western style diet, the daily ω3 PUFA dose requirement is not always ensured.

Despite intensive investigation devoted to the achievement of an adequate dietary intake of ω3 PUFA, more recently, attention has also been focused towards the effects of different ω3 PUFA dietary

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forms (i.e., ethyl esters or PLs) (Neubronner et al., 2011). Within this context, fish PLs have created a great deal of interest (Burri, Hoem, Banni, & Berge, 2012; Dasgupta & Bhattacharyya, 2007; Shirouchi et al., 2007) since they have shown to be more efficient carriers of ω3 PUFA than fish TAGs in terms of ω3 PUFA absorption in different tissues (Parmentier, Al Sayed Mahmoud, Linder, & Fanni, 2007). This is particularly true when ω3 PUFA, such as eicosapentaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), are mainly esterified at the *sn*-2 position, as observed in marine PLs. In addition, fish PLs have also exhibited anti-inflammatory and antitumor-promoting effects.

Sardine (*Sardina pilchardus*) is one of the most commercialized and consumed fishes in the Mediterranean area (Ismea, 2012, pp. 2–5). Sardine lipids have important nutritional characteristics because of their particularly high level of ω3 PUFAs, thus allowing the recommended dietary intake of PUFAs to be easily reached consuming less than three serving of this kind of fish per week and without the need for any supplementation (Bandarra, Batista, Nunes, Empis, 1997; Cardenia, Rodriguez-Estrada, Baldacci, & Lercker, 2013; Pacetti, Balzano, Colella, Santojanni, & Frega, 2013; Pacetti, Mozzon, Lucci, & Frega, 2013). However, most fish are consumed cooked, and considering that the culinary processes undoubtedly alter the content, the composition and the biological activity of the fish lipids, the nutritional value of the final cooked product is of major importance for human health (Nomikos, Karantonis, Skarvelis, Demopoulos & Zabetakis, 2006). Within the wide range of available cooking procedures, deep-fat frying is one of the most common food processing methods used for preparing a worldwide variety of foods, including fish. During frying both oil and food are modified, with ω3 long chain PUFAs in PLs representing the most heat-labile and oxidation-sensitive fatty acids. Over the past 30 years, several studies were undertaken to determine the effects of deep fat-frying and pan-frying on the fatty acids of fish species (Ansorena, Guembe, Mendizabai, & Astiasaran, 2010; Candela, Astiasarán, & Bello, 1998; Gladyshev, Sushchik, Gubanenko, Demirchieva, & Kalachova, 2007; Sanchez-Muniz, Viejo, & Medina, 1992; Sebedio, Ratnayake, Ackman, & Prevost, 1993; Sioen et al. 2006; Weber, Bochi, Ribeiro, & Victório, 2008; Zervou et al., 2012; Zhang et al., 2013). However, despite the increased interest in the nutritional and biological activities of ω3 PUFA rich PLs, there are no current data regarding the modifications of PLs profile that occur during the deep fat-frying procedure. Therefore, the aim of our work was to determine the effects on PL composition of edible muscle (fillet) of *S. pilchardus* of deep-fat frying performed using different culinary fats (extra virgin olive oil, conventional sunflower oil and high-oleic sunflower oil) and different temperatures (160 and 180 °C). The PL molecular species of the main fish PL classes (phosphatidylethanolamine, PE, phosphatidylcholine, PC) were determined by high pressure liquid chromatography (HPLC) coupled with a second order mass spectrometer (MS–MS) with electronebulization interface (ESI).

Beyond providing new information about the effect of the frying process on the ω3 PUFA rich PLs composition, this work helps to clarify the influence of different culinary fats and frying temperatures on the evolution of the PLs fraction, thus providing consumers and food industry with additional knowledge that can be used for the control and/or the preservation of the fried fish quality.

2. Materials and methods

2.1. Material

Chloroform and methanol were HPLC grade from Lab-Scan (Dublin, Ireland); ammonia solution (30%) of analysis grade was from Carlo Erba (Milano, Italy). All other chemicals, with noted exceptions, were obtained from Sigma Chemicals Co. (St. Louis, MO). PLs standards including 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE, purity > 99%), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocoline (DPPC, purity > 99%), 1-palmytoil-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC, purity > 99%), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS, purity 99%), L-α-phosphatidylinositol ammonium salt from bovine liver (PI, purity 98%), 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA, purity 99%), 1-oleoyl-*sn*-glycero-3-phosphocoline (OLPC, purity 99%), 1-palmitoyl-*sn*-glycero-3-phosphocoline (PLPC, purity 99%) were purchased from Sigma (St. Louis, MO, 95 USA).

2.2. Sampling

Sardines (*S. pilchardus*) from the Adriatic Sea were selected and purchased in local fish markets in Ancona (Italy). Whole fishes were immediately beheaded, eviscerated and filleted without removing skin. The obtained fillets (edible muscle) were used for the frying tests which were performed at different temperatures and by using different culinary fats. In the first step, the frying tests in extra virgin olive oil (OO), conventional sunflower oil (SO) and high-oleic sunflower oil (HSO) at 160 °C was carried out. Then, because of the results obtained using different types of oils, the frying test at 180 °C was exclusively performed in oleic sunflower oil (HSO); anyway, this temperature is below the smoke point of all the oils mentioned. Three replicates were performed for each cooking condition (frying oil type and temperature). In each replicate, ten fish fillets (for a total of about 150 g), were introduced into a deep fryer (capacity 2 L), in a closed environment, for 5 min; the oil used was changed after each replicate. The oil temperature prior to start frying has been set to established value and it was controlled by a specific digital thermometer. Furthermore, the evolution of quality parameters (acid and peroxides values, ultraviolet specific absorbance, oxidative/oil stability index – Rancimat Test) of each oil, was monitored during the frying process, as already reported in a previous work (Frega, Strabbioli, Boselli, & Pacetti, 2011).

2.3. Lipid extraction

The lipid fraction from fried fillets was extracted using the method of Bligh and Dyer (1959).

2.4. HPLC-MS/MS analysis of phospholipid molecular species

The total lipids were dissolved in chloroform–methanol (2:1, v/v), in order to obtain a 2–3 mg/mL solution which was injected into the HPLC system. HPLC-ESI-MS/MS was carried out using a pump module (Jasco PU-980) and a ternary gradient module (Jasco LG-980-02, Tokyo, J). The column was a Polaris Si-A 3 μ 150 mm × 4.6 mm (Varian, Middelburg, NL) protected with a Silica precolumn (4 mm × 3.0 mm ID) from Phenomenex (Torrance, USA). A gradient of solvent A [$CHCl_3$/MeOH/NH_4OH (30%) 70:25:1, v/v], and solvent B [$CHCl_3$/MeOH/H_2O/NH_4OH (30%) 60:40:5.5:0.5, v/v] was used. The gradient started at 100% of A, decreased to 0% A (100% B) in 10 min, then was held for 15 min; and then reached back 100% A in 5 min. The flow rate was 1.0 mL/min and the injection loop was 5 μl. The HPLC system was coupled to an LCQ ion-trap mass spectrometer (Finnigan, San José, CA, USA). The mass detector was equipped with an electrospray ionization source (ESI). The steel ionization needle was set at 5.0 kV and the heated capillary was set at 200 °C. The sheath gas flow was approx. 90 arbitrary units. The ion source and the ion optic parameters were optimized with respect to the positive molecular related ions of the phospholipids standards. The molecular mass peaks from the