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Determination of phospholipid molecular species in pork meat by high performance liquid chromatography–tandem mass spectrometry and evaporative light scattering detection

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Abstract

Normal phase high performance liquid chromatography has been optimized for both evaporative light scattering detection and tandem mass spectrometry in order to characterize the natural phospholipids (PL) (classes and molecular species) of raw and cooked pork meat. The PL fraction included phosphatidylcholine (PC) (42.9% ± 4.5 for raw vs 42.6% ± 8.0 for cooked meat), plasmalogen–phosphatidylethanolamine (pPE) and phosphatidylethanolamine (PE) (26.7% ± 3.1 vs 28.5% ± 2.3), cardiolipin (CL) (8.3% ± 2.9 vs 6.3% ± 0.7), sphingomyelin (Sph) (7.5% ± 0.9 vs 8.3% ± 2.1), phosphatidylinositol (PI) (6.8% ± 0.7 vs 6.5% ± 2.1) phosphatidylserine (PS) (4.9% ± 0.5 vs 4.6% ± 1.4) and lysophosphatidylcholine (LPC) (2.9% ± 1.3 vs 3.3% ± 2.6). Arachidonic acid (absent in Sph) was mainly present in pPE and PI and formed molecular species with a saturated fatty acid, such as stearic (as in PI, PS, PE and PC) or palmitic acid (as in PE and PC), or the respective vinyl ethers in pPE (p18:0 and p16:0); however, in PC, arachidonic acid also formed combinations with oleic and linoleic acid. Palmitic acid formed the most abundant molecular species in PC, but not in CL, PE, PI and PS. Unexpectedly, the cooked pork meat showed an increased content of the molecular species of PI and LPC with more unsaturated fatty acids (18:0/20:4 and 18:2, respectively) with respect to raw meat.

Keywords: Pork meat lipids; Phospholipids; High performance liquid chromatography; Tandem mass spectrometry; Evaporative light scattering detection; Fatty acids

1. Introduction

The lipid composition of pork meat is particularly important (Gray, Gomaa, & Buckley, 1996), both for the eating quality of cooked meat and for the quality of many dry-fermented products (ham, sausages, salami) currently available in different countries (Cappelli & Vannucchi, 2005). The lipid composition of meat depends on several factors, such as the animal species, the rearing conditions, the feeding composition and also intrinsic factors, such as the kind of muscle and the kind of muscle fibre. 'Red muscles' usually contain higher amounts of phospholipids (PL) and polyunsaturated fatty acids (PUFAs) with respect to 'white fibres' (Wood et al., 2004). The quantity and quality of the polar lipid fraction (PoL) affects the technological properties (Gandemer, 2002), such as the fat melting point and the rheology of both minced (Chizzolini, Novelli, & Zanardi, 1998) and non-minced (Muriel, Antequera, Petron, Andres, & Ruiz, 2005) products, the emulsion characteristics (Muench, 2004), the oxidative stability (Cascone, Eerola, Ritieni, & Rizzo, 2006) and therefore the shelf life of the finished product, as well as the sensory properties and the nutritional quality (Muguerza, Gimeno, Ansorena, & Astiasarán, 2004). In recent times, the alimentation of domestic livestock has been oriented to a higher content of natural sources of PUFAs, such as grain feeds, due to

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health concerns. This, together with the fact that phospholipids are more susceptible to oxidation processes than the neutral lipids, due to the higher proportion of PUFAs, can determine off-flavours and technological defects in the meat and in cured products (Wood et al., 2003).

The intake of dietary phospholipids is estimated to be around 3–4 g/d, which amounts to about 5–8% of total dietary lipids in many countries (Akoh, 2006). The sales of phospholipids are currently growing in the world due to the applications for food, animal feed, cosmetic/pharmaceutical purposes, chemical products such as insecticides, paint and others. It was also reported that PL can exert therapeutic effects in some neurological disorders and liver cirrhosis (Yanagita, 2003).

Usually, the analysis of phospholipids is based either on the determination of the total fatty acids by gas chromatography after purification of the polar lipid fraction, or the determination of the phospholipid classes (phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, sphingomyelin, lysophosphatidylcholine etc.) with high performance liquid chromatography, as reviewed elsewhere (Peterson & Cummings, 2005).

The aim of this work was to carry out an analytical method for the simultaneous determination of both the phospholipid classes and the phospholipid molecular species within each class in raw and cooked pork meat. After purification of the polar lipid (PoL) fraction by solid phase extraction (SPE), the PL classes were separated with High Performance Liquid Chromatography (HPLC). The chromatographic apparatus was coupled on-line with two detection systems: an ion trap equipped with electrospray ionization for tandem mass spectrometry (MS–MS) and an evaporative light scattering detector (ELSD). These detection techniques were compared both for qualitative and quantitative analysis.

Practically no literature data are available on the modifications of each molecular species of PL after cooking, which play a pivotal role for the study of the change of the nutritional quality of meat. Therefore, the proposed procedure was applied for the characterization of the polar lipids in raw and cooked pork meat.

2. Materials and methods

2.1. Materials

HPLC-grade methanol, chloroform and water were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland). All other reagents were of analytical grade. Cardiolipin sodium salt from bovine heart (98%) and PL standards (purity greater than 99%), including phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) phosphatidylcholine (PC), *N*-palmitoyl-sphingomyelin (Sph) and 1-oleoyl-glycero-3-phosphocholine (lysophosphatidylcholine, LPC) were purchased from Sigma (St. Louis, MO, USA). The samples of pork meat (*Longissimus lumborum muscle*) were supplied by a local distributor. Four replications of each sample (raw and cooked pork meat) were performed. The cooked meat was prepared by heating a 1-cm thick meat slice on a hot iron plate for 5 min. The meat slice was turned upside-down 10 times during heating.

2.2. Extraction and purification of phospholipids

The lipid fraction was extracted according to the method described by Folch, Lees, and Stanley (1957) with slight modification (Boselli et al., 2005). Briefly, the samples of raw or cooked meat were minced and 60 g were homogenized (Ultra Turrax T 20, IKA, Staufen, Germany) with 500 ml of a chloroform:methanol solution (1:1, v/v) in a 1000 mL glass bottle with screw-cap. The bottle was kept at 60 °C for 20 min before adding 250 ml chloroform. After 3 min homogenization, the content of the bottle was filtered through filter paper (Whatman no. 1, Brentford, UK). The filtrate was mixed thoroughly with 200 ml of a 1 M KCl solution and left overnight at 4 °C in order to obtain phase separation. The lower phase was collected and dried by adding 35 g anhydrous sodium sulphate. After filtration on filter paper, the organic phase was dried in a rotary evaporator at room temperature. The lipid extract was stored at −20 °C until the analysis.

The polar lipid fraction was purified by means of Solid Phase Extraction according to Avalli and Contarini (2005). An aliquot (50 mg) of the lipid extract was dissolved in 200 μl of a chloroform/methanol mixture (2:1, v/v) and was applied to a silica gel bonded column (Supelclean LC-Si, 20-ml volume, 5 g sorbents, Supelco Bellefonte, USA). After conditioning with hexane, the non-polar lipids were eluted with 10 ml of hexane–diethyl-ether (4:1, v/v) and 10 ml of hexane–diethyl-ether (1:1, v/v). The recovery of polar lipids was performed with 10 ml of methanol plus 10 ml of a mixture chloroform–methanol–water (3:5:2, v/v/v). The recovered fraction was dried in a rotary evaporator and it was re-dissolved in chloroform–methanol (2:1, v/v), in order to obtain a 7 mg/ml solution to inject into the HPLC system.

2.3. Fatty acid composition of total, non-polar and polar lipids

Fatty acids methyl esters (FAMEs) were obtained from total, polar and non-polar lipids according to the method of Christie (2003). Briefly, the transmethylation of the lipid fractions (50 mg for total lipids, 50 mg for the non-polar lipids, 5 mg for the polar lipid fraction) was achieved in 1% sulfuric acid in methanol. After heating for 2 h in an oven, FAMEs were extracted with *n*-hexane and analyzed. The analysis was performed by means of gas chromatography using a CP-9003 apparatus (Chrompack Middelburg, NL), equipped with a flame ionization detector (FID) and a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 μm, Chrompack). The sample was injected on-column. The carrier gas was