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Polar and neutral lipid composition and fatty acids profile in selected fish meals depending on raw material and grade of products

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ABSTRACT

Fish and fish products are widely distributed feed in aquaculture and agriculture. However, still little is known on the lipid composition of them, potential differences in the lipid profiles of various meals depending on fish composition of meal and process technology. Therefore, the aim of this study was to determine the characteristics of polar and neutral lipids in selected meals. The thirteen fish meals were analyzed using two mass spectrometry technique coupled with gas chromatography and liquid chromatography. The highest lipid content was detected in mixed meal prepared from many species – multi fish meal – (mackerel, trout, sprat, herring, perch, silver carp etc.). In our article for the first time such precise fatty acid profile including atypical acids, e.g. branched fatty acid, was described in fish meals. Polyunsaturated fatty acids (PUFA) dominated in Norsea Mink (Nsm), Mauretania Grade (MG), Human Grade Batch (HGB) and Low Temperature (LT) products, what was associated with the processing technique and whole fish was used for meal production. These products were also abundant in phospholipids. Meals did not subjected to extrusion process and without addition of antioxidant were characterized by low levels of n-3 PUFA and small diversity of polar and neutral lipids.

1. Introduction

Millions of tonnes of fish meal are produced and used in commercial diets for fish, dairy cattle, mink, poultry and swine (Aberoumand, 2010). In fish feed industry exist two types of animal feed, produced from whole fresh industrial fish and from fish offal (Jensen, Fiskeindustri, & Denmark, 1990). In 2008 world production of fish meal amounted about 5 million tons (Penven, Perez-Galvez, & Bergé, 2013) and currently, the supply is stable at 6.0 to 6.5 million tons annually (Miles and Chapman, 2006). In order to produce 1 ton of dry fish meal, 4 to 5 tons of whole fish are required (Miles and Chapman, 2006). Fish meals can be divided into 3 categories: fish meal made from fish, which are not suitable for human consumption (sandeel, Norway pout), fish meal made from fish, which can be consumed by human (blue whiting, sprat, capelin) and fish meal produced from fish, which are commonly consumed by human, but any surplus may be used for fish meal production (herring, mackerel) (Karalazos, 2007).

Balance of amino acids, fatty acids and phospholipids are essential for optimum growth, development and reproduction (Miles and Chapman, 2006; Usydus, Szlinder-Richert, Adamczyk, & Szatkowska, 2011). Until now, authors mainly analyzed content of protein and amino acids composition in fishery products and in commonly available specification of fish products are presented mainly: total amount of protein, fat, water, salt, volatile nitrogen, minerals. Despite application of fish meal in agriculture and aquaculture and beneficial effects of fish meal, we still lack an accurate characteristics of their lipids. Only few previous studies analyzed the profiles of polar (PL) and neutral lipids in fish meal (Vik et al., 2015). Importantly, lipid content and stability of fish meal depends on storage conditions and processing, seasonal variations and fishery location (Samuelsen, Mjøs, & Oterhals, 2014) as well as presence of natural antioxidant (Bragadóttir, Pálmadóttir, & Kristbergsson, 2004). Other conditions, such as species of fish and part of organism intended for meal production will determine the dominant group of lipids (Jensen et al., 1990).

Therefore, the objective of this research was to identify the composition of molecular species of specific lipid classes in fish meal, which is produced in various technology process from whole fish (homogeneous meal) and from by-products of several species

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of fish (mixed meal). Characterization of lipids comprises a new information about the lipid composition in fish products. Two mass spectrometry (MS) technique was used. The total number of FAs was analyzed by using gas chromatography–mass spectrometry (GC–MS) technique with electron ionization (EI). Diacylglycerols (DAGs), triacylglycerols (TAGs), lysophospholipids (LPLs), phospholipids (PLs), ceramides and sphingomyelins were recorded using a liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS^2) technique with Ultra Ion Trap and detector MS via diode array detector (DAD).

2. Material and methods

2.1. Experimental material

Fish meals from several fishing companies were purchased. Research material was divided into three parts: homogeneous meals produced in low temperature process (LT): (1) Blue Whiting, (2) Baltic Sprat, (3) Boar Fish, (4) Capelin and (5) Sandeel; Norsea Mink (Nsm) homogeneous and mixed meals: (6) Sprat, Norway pout and Herring, (7) Sprat and Norway pout, (8) Sprat, (9) Mauretania Grade (MB) Tobias and (10) Human Grade Batch (HGB) Sardinella. The last part of analyzed fish meals were multi meals: (11) before extrusion without antioxidant, (12) after extrusion with antioxidant and (13) after extrusion without antioxidant. Naturox as an antioxidant were added to the all fish meals, except fish meal 11 and 13.

2.2. Chemicals and reagents

Methanol, chloroform, isopropanol, acetonitrile, *n*-hexane, chloric acid, acetic acid, potassium hydroxide, sodium chloride and ammonium acetate were obtained from Avantor (Gliwice, Poland). All the solvents were HPLC grade. Saturated branched chain fatty acid 19-methyl-eicosanoate was used as an internal standard for GC–MS analysis with a 10% BF_3 in methanol, both obtained from Sigma–Aldrich (Poznan, Poland). neutral lipid standards used in the chromatographic analysis were: dilaurin (DAG), trimyristin (TAG); and PLs standards: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (PC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (PE), 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (PS), and sphingosine 1-phosphate (SM) and *N*-Hexanoyl-D-sphingosine (Cer), also obtained from/provided by Sigma–Aldrich (Poznan, Poland). Aminopropyl cartriges Strata™ (500 mg/6 ml matrix) were from Phenomenex (USA).

2.3. Sample preparation

The fish meals were lyophilized and extracted in a chloroform-methanol mixture (2:1, v/v) (Folch, Lees, & Stanley, 1957). The lipid extracts were dried by evaporation under a stream of nitrogen. Each sample was divided into two parts: for the analysis of FA composition by using GC–MS technique and for the analysis of complex lipids, namely polar lipids (ceramides, sphingolipids and phospholipids) and neutral lipids (di- and triacylglycerols) by using LC-ESI-MS^2 technique. The lipid extracts were frozen at −20 °C in glass amber tubes and stored until analysis. The lipid standards were prepared and analyzed according to the identical protocol as the investigated material.

2.4. Spectrometric techniques

2.4.1. GC–MS analysis

FA profiles of the fish meal lipids were determined using GC–MS technique for methyl esters. The lipid extracts were hydrolyzed with 1 mL of 0.5 M KOH in methanol and incubated at 90 °C for 3 h. Subsequently, FAs were extracted by *n*-hexane and the *n*-hexane phase was evaporated to dryness under a stream of nitrogen. Finally, 0.1 mL of 10% BF_3 in methanol was added and the samples were incubated at 55 °C for 1.5 h. The samples were frozen at −20 °C until the GC–MS analysis. The profile of fatty acid methyl esters (FAMEs) was determined using the GC–MS on a QP-2010 SE chromatograph (Shimadzu, Tokyo, Japan) with a 30 m × 0.25 mm i.d. fused silica capillary column Rtx-5 (Restek Corporation, USA) and a 0.25-mm thick film. The temperature of the process was set at 60–300 °C at a 4 °C/min rate, with a 60 kPa helium carrier gas pressure at a column head. Prior to the GC–MS, FAMEs were diluted in dichloromethane.

2.4.2. LC-ESI-MS^2 analysis

Mixture lipids were separated on the lipid group by the solid phase extraction (SPE) on aminopropyl cartridges according to Kaluzny, Duncan, Merrit, and Epps (1985) and Bodennec et al. (2000). The correctness of SPE method and separated lipids groups were confirmed on the Thin Layer Chromatography (TLC) plates (Silica gel 60 F254, 25 Glass plates 20 × 20 cm, without fluorescence indicator, Merck; Darmstadt, Germany). Lipids were analyzed using a LC instrument (Agilent Technologies 1200series, Santa Clara, USA). The Eclipse XDB-C_{18} column (4.6 × 250 mm, 5 μm; Agilent Technologies, USA) was used for chromatographic separation, using the MB phase as an A phase (1 mM ammonium acetate in 90% of water and 10% of acetonitrile, pH 3.7) and acetonitrile with isopropanol (5:2, v/v) as a phase B (with addition of 0.1% acetic acid). The flow rate was set at 0.8 mL/min, and 35 μL of the sample was injected into the column. The column was thermostated at 30 °C. The analysis was conducted on a Bruker Daltonics HCT Ultra Ion Trap (Bremen, Germany). The HPLC system was connected to the MS via DAD (wavelength of λ = 254 nm). The nebulizer pressure was set at 50 psi, dry gas temperature at 360 °C and dry gas flow rate at 11 L/min. The capillary voltage was ± 4 kV. The mass in the MS spectra ranged between 50 Da and 1500 Da, with a 700 Da target mass.

The instrument was run in positive and negative ion mode. The obtained mass spectra represent average values for three scans. Prior to the LC-ESI-MS^2 analysis, the lipid samples were diluted with a mixture of acetonitrile and methanol (1:1, v/v). LC-operation, data acquisition and processing were carried out using a ChemStation for LC systems Rev. B.01.03-SR2 (Agilent Technologies, USA) and esquire Control 6.1 coupled with Bruker Daltonics DataAnalysis 3.4 (Bruker Daltonics, Germany). Specific components representing various phospholipid classes were identified with a SimLipid 4.2 software (PREMIER Biosoft International, USA).

2.5. Statistical analysis

The statistical significance of differences between the groups was assessed by a one-way analysis of variance (ANOVA) and Tukey's post hoc test used for further determination of significance of differences. Differences between the groups were considered significant when $p < 0.05$. All data are presented as means ± SD. The number of individual measurements taken during the GC–MS and LC-ESI-MS^2 analyses was 3. SigmaPlot for Windows version 11.0 software was used for all statistical analyses (Systat Software Inc., Germany).

3. Results

3.1. Lipids content

In our research we analyzed LT, Nsm, MG, HGB grade meals and