Supercritical fluid extraction of fatty acids and phospholipids from whole quail eggs

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The aim of this study was to determine the effect of the addition of selenium yeast and algae on fatty acid profile of the lipid fractions of quail's whole egg. Fatty acid profile of fractions obtained by supercritical carbon dioxide extraction in the pressure range 10 to 40 MPa, and marked in the remains after extraction. Japanese quail (Coturnix coturnix japonica) were kept in two experimental groups in batteries and fed ad libitum. The relation between ω6 and ω3 fatty acids determined for egg triglyceride fraction collected from birds fed with supplemented fodder decreased more than three times comparing to the control fraction. Changes in the relation ω6/ω3 fatty acids polar in the phospholipid fractions of analyzed groups decreased more than twice (α=0.05).

Keywords: Japanese quail; egg; phospholipid; supercritical fluid extraction; carbon dioxide.

Introduction

Supercritical fluid extraction (SFE) is a dynamically developed technique in the last decade. During the SFE process carbon dioxide with the supercritical parameters of temperature 31.05°C, pressure 7.38 MPa, and density 468 kg/m3 is usually used. Carbon dioxide used for the extraction is non-toxic, non-flammable, environmentally friendly, cheap, and after the process completely removed from the collected extracts. Supercritical carbon dioxide has low viscosity and a high degree of diffusion. The selectivity of extraction depends on the density of the fluid that is regulated when changing the critical parameters of temperature and/or pressure (Abbas et al., 2008, Sekhon, 2010). In the food industry the SFE is applied for the extraction of aromas and colours from plant raw materials, decaffeination of coffee and tea, removing alcohol from wine and beer as well as for fractionation of egg yolk eggs esp. for cholesterol reduction and extraction of phospholipids (Brunner, 2005).
Phospholipids are a group of amphiphilic substances, in which hydrophilic (polar) region is located in the sn3 position of the glycerol chain esterificated by phosphoric acid together with choline to form the phosphatidylcholine (lecithin) or with phosphatidylethanolamine (cephaline). In the sn1 and sn2 positions esterificated saturated and unsaturated fatty acids are usually located. Phospholipids are natural emulsifiers used in many branches of the food pharmaceutical industries as well as in the cosmetology. Phospholipids are generally collected from post-extraction sludge during soybean and rapeseed oil refining and they are used on an industrial scale in the food industry. The pharmaceutical industry utilizes more egg yolk phospholipids. Comparing to the soybean, phospholipids from egg yolks contain more than twice phosphatidylcholine (75%), and higher degree of fatty acid unsaturation. Phospholipids colleted during the extraction process under supercritical carbon dioxide conditions are characterized by higher purity, expressed as acetone insoluble matter, compared to classical chemical extraction process (Boselli and Caboni, 2000, Mr *et al.* 2001, Bobak *et al.*, 2013).
The aim of this study was to determine the effect of the addition of selenium yeast and algae on fatty acid profile of quail's whole egg lipid fractions extracted by supercritical carbon dioxide.

Materials and methods

Eggs were collected from Japanese quails (*Coturnix coturnix japonica* J-11) kept in the battery cages in groups of 40 per square meter. Birds from the age of 16 up to 24 weeks were fed *ad libitum*; with a standard mixture Farm line G-090 for quails enriched with the *Saccharomyces cerevisiae* yeast photocells with a concentration of 500 mg selenium Se/kg (0,3 g/kg), DHA Gold (5 g/kg, Novus International, Inc., MO, USA) and flaxseed (40 g/kg). Collected eggs were higienized and then automatically broke using egg shell breaker UDTJ-150 (OVO TECH; Starachowice; Poland) The homogenous whole mass of the quail eggs was dehydrated using the Mobile Minor spray drier (Pvt. a/s; Gladsaxevej; Denmark) with disk atomization system. The parameters of the drying process has been maintained at the level of 185±5°C for inlet air and 90±2°C for outlet air.

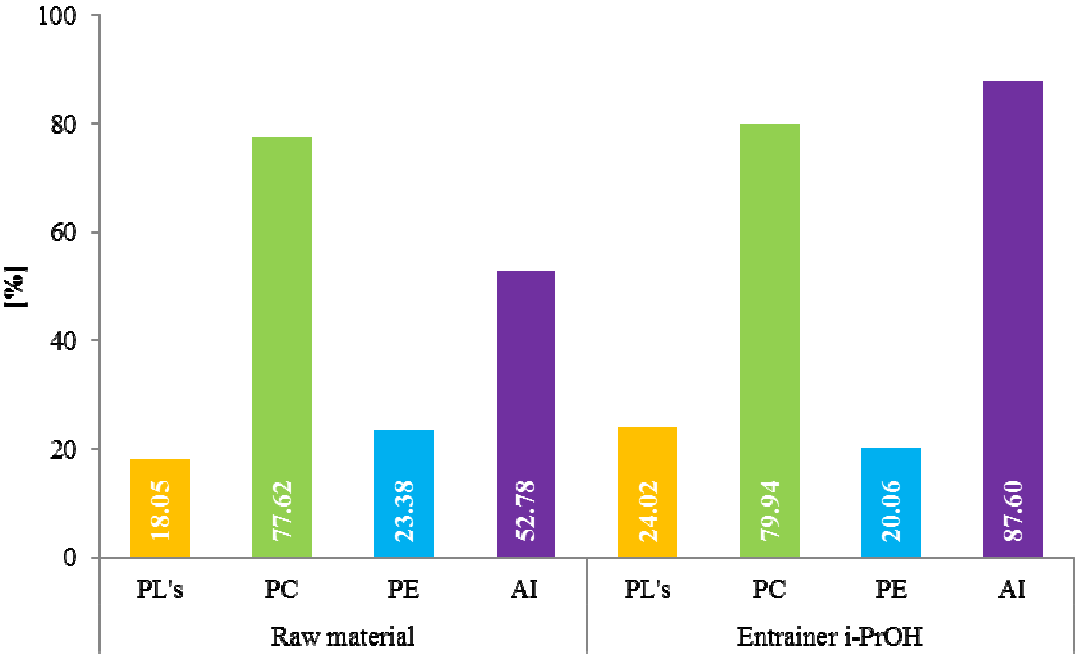
The supercritical fluid (carbon dioxide, $scCO_2$) extraction process (Linde Gas Poland, Kraków) was performed using high pressure extractor with working range up to 100 MPa and temperatures up to 80°C (Natex Prozesstechnologie GesmbH Ternitz, Austria). The process was carried out up to 50 MPa, the operating pressure increased more after the disappearance of the separated components. After the recovery of 40% of the extracted material, 10% of the entrainer - isopropyl alcohol (i-PrOH) (calculated on the flow of gas), was added to carbon dioxide in supercritical state. Extraction and separation processes were carried out at the temperature of 60°C and 40°C, respectively. The separation process was performed at a pressure of 6 MPa, the flow of gas was stabilized at 32 kg CO_2/h (working pressure was increased by increasing the frequency of the plunger pump).

The total lipid content was determined by Folch method (1957) in raw material and residues after extraction. The fatty acid profile in the separated fractions and recovered i-PrOH (after evaporation of the solvent under vacuum - Buchi R215 rotary evaporator) was analyzed after methylation (14% BF_3 in methanol). The analysis was performed in a gas chromatograph with a spectroscopy mass detector (GC6890/5973 MSD, column – HP88). HPLC analysis of PLs was done using HP 1200 system equipped with Diode Array Detector (DAD G1315B) on the Ascentis Si column (150 × 4.6 mm). The HPLC condition flow rate 1 mL/min, phase A - 2% phosphoric acid in acetonitrile, phase B - 2% phosphoric acid in methanol in the ratio of 3:1 (v/v). GC/MS and HPLC/DAD were purchased from Agilent Technologies, Inc.. CA, USA..

The whole egg mass consisted of more than 23% PUFA, in which 3.66% was α-linolenic acid esterificated in triacylgliceride fraction (TAG) (tab. 1). Phospholipids fraction contained esterified eicosapentaenoic and docosahexaenoic acids, which were not extractable by fluid carbon dioxide. The application of the entrainer (10% i-PrOH on the flow gas) to the fluid gas enabled to increase the field of phospholipids containing EPA and DHA, app. 53% of the total PLs in the raw material (fig. 1 The collected results different from in to those reported by Boselli and Caboni (2000). The content of PC was higher in lipid fractions obtained during SFE processing. Phospholipid fractions after supercritical fluid extraction were characterized by the purity higher of about 30% comparing to the chemical extraction (CE). Saturated fatty acids were present in the raw material in the amount of 38.33%, and were comparable to Kaźmierska et al. (2007). Moreover, raw material contained 5.55% *n*-3 fatty acids in the lipid fractions and 7.69% in the phospholipids fractions. The contents of *n*-6 fatty acids were statistically different (p=0,05) and the average values were 16.42% and 17.55% for lipid and phospholipids fractions, respectively. Gładkowski et. al. (2013) reported that the content of *n*-3 fatty acids in the quails egg fed with suplemented mixture was 7.33%, thus this results were comparable to those collected in our study.

The ratio between *n*-6 and *n*-3 fatty acids in the phospholipids extracted from raw materials was 2.14, whereas in the residues 3.16. In the phospholipids extracted with the application of entrainer the ration was significantly lower (1.52).

Figure 1 The composition of phospholipid fractions



PL's – phospholipids; PC- phosphatidylcholine; PE – phosphatidylethanolamine; AI - acetone-insoluble matter

Table 1 Content of fatty acids in phospholipids and fat fraction in the raw material and residual after extraction, %.

Variant		Extraction	SFA	MUFA	PUFA	*n*-3	*n*-6	n-6/n-3	ALA	EPA	DHA
Raw material		Alkohol	49.30fg	20.85b	29.85i	7.69k	16.42c	2.14b	1.01ef	0.61h	6.07i
		Folch	38.33b	38.51h	23.16e	5.55g	17.55d	3.16d	3.66j	0.24c	1.79c
SFE	Pure fluid - CO_2		35.08a	44.70i	20.22c	3.75d	16.39c	4.37e	3.75k	0.00a	0.00a
	Entrainer	i-PrOH	55.32k	26.88f	17.80a	7.07j	10.73a	1.52a	1.30h	0.77i	5.00g
	Residue after extraction	Alkohol	34.85a	29.12g	36.01l	8.67m	27.36c	3.16d	0.73c	0.56g	6.26j
		Folch	48.20d	21.02b	30.78j	8.03	22.42f	2.79c	1.04f	1.40e	5.74h

Means with the same letters are not significantly different at p = 0.05.

Conclusions

Summing up, it can be concluded that quail eggs can be enriched in PUFA, especially EPA and DHA, by the modification of the fodder composition. Supercritical fluid extraction with carbon dioxide and entrainer application can be used to fractionation the egg materials focusing on phospholipids recovery. Purified PLs after SFE process were characterized by higher purity, expressed as AI, and enhanced fatty acids profile, comparing to CE. High purity of the phospholipids enables applications of this fraction in the pharmaceutical industry, among others for the liposome formation.

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