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The development of a choline rich cereal based functional food: Effect of processing and storage

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ABSTRACT

Based on a growing need for additional dietary sources of choline, this research developed a choline rich cereal based functional food. Egg yolk lipids were extracted using ethanol to produce an egg yolk extract (EYE) of high choline content. Corn meal, wheat flour, sugar, salt and EYE were mixed and extrudates made using a twin-screw extruder with an exit temperature of 120 °C. Products were packaged and stored at −20 °C and room temperature for 12 weeks. Initial processing decreased the total fat and total choline content by up to 30% and 19% respectively. Similarly, tocopherols, tocotrienols and cholesterol decreased after processing. However, the total fat and choline content and tocopherols and tocotrienols did not significantly change during the storage stability study. A shelf-stable choline rich (115 mg/30 g serving) functional food product was developed that could provide a way for consumers to meet their choline requirement.

1. Introduction

Choline has been recognized by the Institute of Medicine as an essential nutrient since 1998, and dietary recommendations set an Adequate Intake (AI) value (Institute of Medicine, 1998, pp. 390–422). It is essential for liver and brain function, lipid metabolism and transport, cell membrane signalling, cell composition and repair (Dowhan, 1997; Zeisel & da Costa, 2009). Although consumption of very high amounts of choline, particularly phosphatidylcholine (PC), have been associated with higher plasma concentrations of trimethylamine-oxide (TMAO) a potential cardiovascular risk factor (Wang et al., 2011), dietary studies have consistently reported that the intake of choline by the population is below what is considered optimal (Lewis et al. 2014; Yonemori et al., 2013). This suggests that new dietary sources of choline are needed. Choline is available in the diet in a number of forms (Patterson, Bhagwat, Williams, Howe, & Holden, 2008, pp. 12–37), with the majority being present as free choline and phosphatidylcholine (Lewis et al. 2014). Eggs are a particularly high source of phosphatidylcholine and we found that egg consumers were more likely to meet daily recommendations for choline (Lewis et al., 2014). Based on this observation, a functional food using eggs could provide an acceptable source of choline for the consumer.

Egg yolk is particularly rich in phospholipids (PL), comprising approximately 10% of the wet weight of the egg yolk, equivalent to about 22% of the total egg yolk solids and 50% of lipids (Palacios & Wang, 2005). The main component of egg-yolk PL is PC, accounting for 80% to the total weight (Chojnacka et al., 2014). PL are used in several industrial applications, e.g. as emulsifiers in pharmaceutical and food products and components of liposomes for cosmetics and drug delivery (Dowhan, 1997).

Extrusion cooking is one of the most important food processing technologies that is used for the production of breakfast cereals, ready-to-eat snack foods, and other textured foods (Brennan, Brennan, Derbyshire, & Tiwari, 2011). The use of extrusion cooking has several advantages over conventional cooking/processing techniques such as improved digestibility and nutrient bioavailability (Gu, House, Rooney, & Prior, 2008) in addition to being versatile, having high productivity, low operating costs, energy efficiency and shorter cooking times (Brennan et al., 2011; Hirth, Leiter, Beck, & Schuchmann, 2014).

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The regular consumption of breakfast cereals has been associated with lower stress and better reported physical and mental health and a good indicator of a healthy lifestyle (Lattimore, Walton, Bartlett, Hackett, & Stevenson, 2010; Smith, 2003; Williams, 2014; Witbracht, Keim, Forester, Widaman, & Laugero, 2015). However, it should be noted that these studies were mostly subjective reports of outcome measures.

The objective of this research was to develop a choline rich extruded breakfast cereal product, which may assist consumers in meeting their recommended choline intake, particularly for non-egg consumers and individuals and those individuals with higher choline needs/requirements such as during pregnancy and lactation. In addition, the effect of processing and storage on the retention and stability of choline was studied.

2. Materials and methods

2.1. Materials

Chicken eggs, corn meal, wheat flour, sugar and salt were purchased from local grocery stores. Anhydrous ethanol was purchased from Greenfield ethanol Inc. (Brampton, Ontario, Canada). Tocopherol standards (>99%), cholesterol standard (>99%), L-α-Phosphatidylcholine (from egg yolk, ≥99%), sphingomyelin (from egg yolk, >95%), choline chloride (>98%), choline-trimethyl-d9 (Cho-d9) chloride, phosphocholine chloride calcium salt tetrahydrate (Sigma grade), betaine hydrochloride (>99%) were purchased from Sigma (St. Louis, MO, USA); 1,2-distearoyl-sn-glycero-3-phosphocholine-*N*,*N*,*N*-trimethyld9 (PC-d9), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-*N*-methyl (16: 0 monomethyl-PE, MMPE), and L-α-lysophosphatidylcholine (egg, chicken) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Glycerophosphocholine was supplied by Bachem Americas Inc. (Torrance, CA, USA). Phosphocholine-*N*,*N*,*N*-trimethyl-d9 (Pcho-d9) chloride calcium salt was purchased from C/D/N Isotopes, Inc. (Quebec, Canada). All other chemicals of analytical or HPLC grade were purchased from Fisher Scientific (Ottawa, ON, Canada).

2.2. Preparation of egg lecithin (egg yolk extract (EYE))

Egg lecithin was prepared following the method described by Palacios and Wang (Palacios & Wang, 2005). Briefly, the shells of fresh eggs were carefully broken and the yolks were separated from the whites. This was achieved by first decanting the whites then carefully rolling the yolks on filter papers to completely remove the whites. The yolks were pooled into a 6 L three-neck flask and anhydrous ethanol was added at 1:6.45 yolk to ethanol mass ratio and stirred for 2 h at 450 rpm using a motorized stirrer. The flask was then stored at 4 °C overnight to precipitate proteins. The precipitate was then removed by filtration and anhydrous Na_2SO_4 was added to the supernatant. The Na_2SO_4 was then removed by vacuum aided filtration. The ethanol was removed from the filtrate using a rotovapor (Laborota 4010, Heidolph, Schwabach, Germany). The egg yolk extract (EYE) was stored at −20 °C for later use.

2.3. Extrusion processing

The ingredients list and amounts are shown in Table 1 for the feed mix used for extrusion. The individual ingredients, with the exception of EYE, were weighed into a large stainless steel mixing bowl. A sample of the ingredients was then moved into the mixing bowl of a high speed small laboratory mixer (Robot Coupe R2 mixer, Ridgeland, MS, USA) and the EYE was added and mixed for about 3 min. The EYE mix was then added to the content of the large stainless steel mixing bowl and mixed for about 10 min using a large laboratory mixer (Hobart mixer, Troy, OH, USA). The control feed mix was treated the same way as the EYE-fortified feed without the high speed mixing step.

Table 1
Ingredient composition used for feed mix preparation for extrusion runs.

Ingredient	Amount (%)	
	Control	EYE-fortified
Corn meal	85	80.69
Wheat flour	12.25	11.63
EYE	0	5.07
Sugar	2.5	2.37
Salt	0.25	0.24

Extrusion runs were conducted at the Food Processing Development Centre of the Alberta Agricultural and Rural Development in Leduc and Brooks Alberta. A Coperion Werner & Pfleiderer ZSK-26 MC (Stuttgart, Germany) co-rotating twin screw extruder was used for the trials. The barrel length to diameter ratio is 40 mm with an overall barrel length of 1040 mm. The screw housing consists of ten barrel sections and a die holder plate. With the exception of the first one, each section was separately heated as follows: sections 2–4 at 50 °C, sections 5–8 at 95 °C, section 9 at 115 °C while the last section was at 120 °C. The die had two circular holes with a diameter of 3 mm. The screw diameter was 25 mm and the ratio of the inner to the outer diameter was 1.55. After extrusion, products were dried in a convection oven at 70 °C to decrease moisture content to below 10%. Products were packaged in transparent low-density polyethylene (LDPE) bags (33 cm × 25 cm × 0.2 mm) and sealed. Samples were stored at −20 °C and at room temperature with opened and closed bags for 12 weeks.

2.4. Analytical

2.4.1. Moisture content determination

Moisture content of the extruded product was determined gravimetrically after lyophilisation. All analyses were conducted in triplicates unless otherwise noted.

2.4.2. Total choline determination

Extraction and determination of phospholipids and choline containing compounds were performed following procedures reported elsewhere (Xiong et al., 2012).

2.4.3. Total fat determination

Total fat content of feed mix and extrudates were determined according to AOAC International method 996.01 (AOAC, 2000) as modified by Robinson et al. (Robinson, Singh, & Kays, 2008).

2.4.4. TBARS determination

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by (Buege & Aust, 1978), and modified by (Lee, Hendricks, & Cornforth, 1999). Approximately 1 g samples of finely ground feed mix and extruded products were placed in glass tubes and mixed with 5 ml of stock solution containing 0.375% thiobarbituric acid, 15% trichloro-acetic acid and 0.25 N HCl. The mixture was heated for 10 min in a boiling water to develop colour. Tubes were then cooled to room temperature under running tap water and centrifuged at 5500 rpm for 10 min. The absorbance of the supernatant was measure at 532 nm against a blank containing all the reagents without the sample.

2.4.5. Tocopherols, tocotrienols and cholesterol determination

Samples were prepared for analysis using the method described