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Lipid composition and emulsifying properties of canola lecithin from enzymatic degumming

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ABSTRACT

This study investigated the polar lipid composition and emulsifying properties of canola lecithin from enzymatic degumming (CLED). Phospholipase A_1 was used for enzymatic degumming of crude canola oil to collect lecithin sample. Canola lecithin from water degumming (CLWD) was also collected and served as the control. The results showed that the contents of phosphatidylethanolamine (PE) (2.99%) and phosphatidylcholine (PC) (6.59%) in CLED were significantly lower than that in CLWD (PE 15.55% and PC 21.93%); while the content of lysophosphatidylcholine (LPC) (19.45%) in CLED was significantly higher than that in CLWD (3.27%). Unsaturated fatty acids accounted for a higher percentage of the total fatty acids in CLED than in CLWD. CLED promoted more stable o/w emulsions than CLWD. This study provides a better understanding of the chemical nature of CLED, and important information for utilization of CLED as o/w emulsifier.

1. Introduction

Lecithin is a mixture of phospholipids (PL), lysophospholipids (LPL), glycolipids (GL), triglycerides, carbohydrates and other minor components (Van Nieuwenhuyzen & Tomás, 2008). In food industry, lecithin is commonly used as emulsifier due to the presence of surface-active polar lipids: PL, GL and LPL. PL is a group of lipids which consist of two fatty acids, a glycerol unit and a phosphate group which is esterified to a simple organic molecule (Szuhaj & Gary, 1985). GL refers to a group of lipids comprised of one or more monosaccharide moieties and a hydrophobic moiety such as an acylglycerol, a sphingoid, a ceramide or a prenyl phosphate, which are linked to the saccharide moieties through a glycosidic bond (IUPAC-IUBMB, 1997). LPL is produced by removing one of the fatty acids from a PL molecule (D'Arrigo & Servi, 2010). All the three groups of lipids, PL, GL and LPL, are amphiphilic, since they contain both hydrophilic and hydrophobic moieties in their structures.

Lecithin is produced from gum generated during the degumming step of vegetable oil refining process. The degumming step is designed to remove PL from crude vegetable oil in order to improve the quality of the final oil product and facilitate the following refining steps: neutralization, bleaching and deodorization (Flider, 1985). Water and acid degumming are the two conventional methods used to degum vegetable oils. Lecithin is most commonly produced from water degumming. Recently, enzymatic degumming which utilizes enzymes to hydrolyze phospholipids, has been adopted by a few vegetable oil refiners due to the increased oil yield (Galhardo & Hitchman, 2012). Phospholipase A_1 (PLA_1) and phospholipase C (PLC) are the two enzymes mostly used in the industry. PLA_1 hydrolyzes PL into LPL and free fatty acid (Merkel et al., 1999), while PLC hydrolyzes PL into diacylglyceride and phosphate ester (Wang, 2001). Although enzymatic degumming has been investigated (Dahlke, 1997; Galhardo & Dayton, n.d.; Jahani, Alizadeh, Pirozifard, & Qudsevali, 2008), and successfully applied in the industry, the gum from this process has not been used for lecithin production. This is mainly due to the limited information available on the chemical composition and properties of lecithin obtained from enzymatic degumming.

Lecithin can be obtained from different oilseeds such as soybean, sunflower seed, and rapeseed (Szuhaj, 2005). As an edible cultivar of rapeseed and one of the leading oilseeds, canola is also a source for lecithin production. The objectives of this study were to investigate the polar lipid composition and emulsifying properties of canola lecithin from enzymatic degumming. Canola lecithin obtained from the conventional water degumming was used as the control.

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2. Materials and methods

2.1. Materials

Crude canola oil was provided by ADM (Decatur, IL). Phospholipase A_1, Lecitase Ultra, was donated by Novozymes (Bagsvaerd, Denmark). This enzyme has a declared activity of 10,000 LU/g (LipaseUnit/gram). One LU is defined as the amount of enzyme that releases 1 μmol of titratable butyric acid from tributyrin substrate in 1 min at 40 °C with pH 7. Polar lipid standards acylated sterol glucosides, sterol glucosides, cerebrosides, monogalactosyl diglycerides and digalactosyl diglycerides were purchased from Matreya (Pleasant Gap, PA). Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol (sodium salt), phosphatidic acid (sodium salt), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidic acid (sodium salt), and lysophosphatidylinositol (sodium salt) were purchased from Avanti (Alabaster, Alabama). Acetone (ACS grade) was from Pharmco-AAPER (Brookfield, CT). Chloroform and methanol (HPLC grade) were from EMD Millipore (Cincinnati, OH). A standard mixture of fatty acid methyl esters (FAME) was purchased from Supelco (Supelco 37-component FAME mix, Supelco, Bellefonte, PA). Undecanoic acid (11:0) (99% GC grade) was from Sigma-Aldrich (St. Louis, MO).

2.2. Recovery of canola lecithin

Canola lecithin was recovered from crude canola oil by enzymatic degumming with Lecitase Ultra, as described by Xie and Dunford (2015). Crude canola oil, 1500 g, was placed in a 2 L jacketed glass reactor, which was connected to a refrigerated/heated circulating bath (Model 11679, VWR, Bristol, CT, USA). The oil was heated to 80 °C and 1.95 g 50% (wt.%) citric acid solution was added. The oil and citric acid mixture was homogenized using an Omni homogenizer (GLH, Kennesaw, GA, USA) with a 20 mm × 195 mm saw tooth generator probe at 24,000 rpm for 1 min. The resulting mixture was stirred at 80 °C and 500 rpm for 20 min using an overhead stirrer (Eurostar, IKA, Wilmington, NC, USA). After cooling the mixture to 50 °C, 1.90 g 4 N NaOH solution was added followed by the addition of 0.06 g enzyme product (40 ppm) and 34.9 g deionized water. The total amount of water used was 2.5% based on the weight of oil. The mixture was homogenized at 24,000 rpm for 1 min, and then stirred at 50 °C and 500 rpm for 6 h. After enzymatic reaction, the temperature of the mixture was increased to 80 °C and kept at this temperature for 0.5 h to deactivate the enzyme. The mixture was then centrifuged using a floor type centrifuge (Sorvall RC 5C, Thermo, Ashwville, NC, USA) at 1366.2*g* for 5 min to separate the wet gum from the degummed oil. Lecithin was prepared by washing the wet gum with cold acetone according to AOCS official method Ja 4–46 (AOCS, 2003), and was kept at −20 °C until further use and analysis.

Water degumming of crude canola oil was also performed. Crude canola oil, 1500 g, was heated to 80 °C. After 2.5% of deionized water addition the oil-water mixture was stirred at 80 °C and 500 rpm for 1 h. The mixture was then centrifuged at 1366.2*g* for 5 min to separate the wet gum from the oil. The wet gum was treated with cold acetone according to AOCS official method Ja 4–46 (AOCS, 2003) to collect lecithin. This lecithin from water degumming was used as the control for the lecithin from enzymatic degumming.

2.3. Polar lipid analysis

PL, LPL and GL contents of canola lecithin samples were determined by HPLC-ELSD, which was based on the method used by Sugawara and Miyazawa (1999), with modification. The HPLC system was Alliance 2695 (Waters Corp., Milford, MA). The Evaporative Light Scattering Detector (ELSD) was Alltech 2000 (ALL Tech Associates Inc., Deerfield, IL). The conditions for ELSD were: nitrogen flow rate of 3.5 L/min, impactor ON, and drift tube temperature of 80 °C. A μPorasil silica column (125 Å, 10 μm, 300 mm × 3.9 mm id., Waters, Milford, MA) was used. The mobile phase consisted of A: chloroform and B: methanol/water (95:5, v/v). The elution program for a binary gradient program was as follow: 0–15 min, 99–75% A and 1–25% B; 15–20 min, 75–10% A and 25–90% B; 20–25 min, 10% A and 90% B; 25–30 min, 10–99% A and 90–1% B. The column was kept at 30 °C, and the mobile phase flow rate was 1 mL/min. The samples were dissolved in chloroform/methanol (2:1, v/v) to achieve a concentration of 20 mg/mL, and filtered through a 0.45 μm syringe filter prior to injection. Sample injection volume was 20 μL. External standard curves were constructed to quantify different polar lipids in samples. The concentrations of the glycolipid standard solutions were 0.1–1 mg/mL, and the concentrations of phospholipid and lysophospholipid standard solutions were 0.5–5 mg/mL.

2.4. Fatty acid analysis

Fatty acid composition of canola lecithin samples were analyzed by GC-FID. The lecithin samples were first methylated according to the AOCS official method Ce 2–66 (AOCS, 2003) before injection to GC column. The GC unit was an Agilent Technologies model 6890 system coupled with a flame ionization detector (FID) (Palo Alto, CA). A Supelco SP-2560 fused silica capillary column, 100 m × 0.25 mm × 0.2 μm film thickness (Bellefonte, PA) was used for the analysis. Helium was used as the carrier gas at a flow rate of 20 mL/min. The injector temperature was kept at 260 °C. The GC oven temperature program was as follows: 140 °C for 5 min, 140 °C to 240 °C at a rate of 4 °C/min, 240 °C for 5 min. The detector conditions were as follows: temperature of 260 °C, hydrogen gas flow rate of 10 mL/min, air flow rate of 450 mL/min, and make-up gas (Helium) flow rate of 45 mL/min. Methylated sample, 1 μL, was injected into GC by an auto sampler (HP 7683, HP Company, Wilmington, DE). A split ratio of 100:1 was used. Standard mixture of fatty acid methyl esters (FAME) was used for peak identification and quantification. Undecanoic acid was used as the internal standard.

2.5. Preparation of o/w emulsions

O/w emulsions were prepared using a commercial canola oil (Crisco, The J.M. Smucker Company, Orrville, OH) as the oil phase. Deionized water and Na_2HPO_4–NaH_2PO_4 buffer solution at pH 7.5 were used as the aqueous phase. Lecithin samples were used as the emulsifiers. The ratio of oil to aqueous phase was 1/9 (w/w), and the total weight of emulsion was 20 g. The emulsifier used was 1% based on the weight of oil. The emulsifier was dissolved in oil or aqueous phase first, and then mixed with the other phase. The mixture was homogenized at room temperature using the Omni homogenizer with 10 mm × 195 mm probe at 24, 000 rpm for 1 min.

2.6. Characterization of o/w emulsions

A pH/Conductivity Meter (AR 20, Fisher Scientific, Pittsburgh, PA) was used to measure the pH of o/w emulsions at room temperature. For particle size measurement, o/w emulsions were diluted with the aqueous phase to an oil droplet concentration of about 0.005% (wt.%). The particle size distribution of the emulsions was determined using a High Performance Particle Sizer (HPPS 5001, Malvern Instruments, Worcestershire, UK). The volume