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Lipid composition and emulsifying properties of canola lecithin from degumming with phospholipase A_2 and its ethanolic fractions

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ABSTRACT

Recovery and chemical properties of lecithin from enzymatic degumming of vegetable oils with phospholipase A_2 (PLA_2) have not been reported to date. The aim of this study was to investigate the lipid composition and o/w emulsifying properties of lecithin obtained from degumming of crude canola oil with PLA_2 (CLP) and its ethanolic fractions, in order to exploit their potential applications in industry. Phosphatidylinositol + phosphatidic acid + lysophosphatidylinositol (30.56 g/100 g), lysophosphatidylcholine (17.41 g/100 g) and phosphatidylcholine (10.17 g/100 g) were the three major lipid groups found in CLP. The ethanol soluble and insoluble fractions of CLP contained lysophosphatidylcholine (45.25 g/100 g) and phosphatidylinositol + phosphatidic acid + lysophosphatidylinositol (31.41 g/100 g) as the most abundant lipid group, respectively. CLP and its ethanolic fractions were superior to a commercial soy lecithin as o/w emulsifiers under the conditions investigated. This study demonstrated that the ethanol soluble fraction of CLP, which contained an abundant amount of lysophosphatidylcholine, would be an ideal starting material for lysophosphatidylcholine recovery, and CLP and its ethanolic fractions can be utilized as o/w emulsifiers.

1. Introduction

Lecithin is a mixture of phospholipids, lysophospholipids, glycolipids, triglycerides, carbohydrates and other minor components (Nieuwenhuyzen & Tomás, 2008). It is produced from gum generated during the degumming step of vegetable oil refining process. The degumming step is designed to remove phospholipids from crude vegetable oil to improve the quality of the final 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 obtained from the conventional degumming processes usually contains four major phospholipid groups: Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid. Due to the different solubility of various phospholipids in ethanol, fractionation of lecithin with ethanol is commonly employed to separate lecithin into ethanol soluble and insoluble fractions, which are enriched in phosphatidylcholine and phosphatidylinositol, respectively (Prosise, 1985). Lecithin and its fractions are usually utilized as emulsifiers in food industry due to the amphiphilic nature of phospholipids, lysophospholipids, and glycolipids.

In the past two decades, enzymatic degumming which utilizes enzymes to hydrolyze phospholipids has been developed for the increased oil yield (Galhardo & Hitchman, 2012). Phospholipase A_2 (PLA_2) was the first and most studied enzyme for enzymatic degumming of vegetable oils (Dahlke, 1997; Gofferjé et al., 2014; Mao, Wei-Lin, Zi-Niu, Ke-Ren, & Liu, 2007; Mukherjee, Monda, Mondal, & Mondal, 2013; Yu et al., 2014), which hydrolyzes phospholipids into lysophospholipids and free fatty acid (Merkel et al., 1999). Although enzymatic degumming with PLA_2 has been extensively investigated, lecithin has not been recovered from the gum generated during this process, and also has not been investigated for its potential utilization as emulsifier.

Lecithin can be obtained from different oilseeds such as soybean, sunflower seed, and rapeseed (Szuhaj, 2005). Canola is an edible cultivar of rapeseed and one of the leading oilseeds. It is also a good source for lecithin production. The objective of this study

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was to investigate the lipid composition and oil-in-water (o/w) emulsifying properties of canola lecithin obtained from enzymatic degumming using PLA_2. In addition, the lecithin fractions obtained by ethanol fractionation were also investigated.

2. Materials and methods

2.1. Materials

Crude canola oil was provided by ADM (Decatur, IL). Lysomax was donated by Danisco (Copenhagen, Denmark). It was produced from a selected strain of *Bacillus licheniformis*, and exhibited phospholipase A_2 activity. The declared activity for this enzyme is 15×10^{-6}–18.33×10^{-6} kat/g. One Katal is defined as the amount of the enzyme that catalyzes the conversion of 1 mol of substrate (lecithin) into 2-lysolecithin per second at 37 °C and pH 8.0. Glycolipid 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) and absolute ethanol (100%) were from Pharmco-AAPER (Brookfield, CT). Chloroform and methanol (HPLC grade) were from EMD Millipore (Cincinnati, OH).

2.2. Recovery of canola lecithin

Canola lecithin was recovered from crude canola oil by enzymatic degumming with Lysomax, according to the procedure 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 circulating water bath (Model 11679, VWR, Bristol, CT, USA). The oil was heated to 80 °C and 1.95 g citric acid solution (50 g/100 g) 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 NaOH solution (4 mol/L) was added followed by the addition of 0.06 g enzyme (40 mg/kg) and 34.9 g deionized water. The total amount of water used, including the amount in the citric acid solution (0.975 g) and NaOH solution (1.625 g), was 25 g/kg 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 1367*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 then kept at −20 °C until further use and analysis.

2.3. Fractionation of canola lecithin

Canola lecithin, 2.5 g, was mixed with 15 mL absolute ethanol with a magnetic stirrer (Spin Master 4802, Cole-Parmer, Vernon Hills, IL) at 50 °C for 0.5 h. The mixture was centrifuged at 2991*g* for 5 min. Ethanol soluble and insoluble fractions were separated and dried under vacuum at 80 °C. Dried fractions were stored at −20 °C until further analyses.

2.4. Lipid analysis

Phospholipid, lysophospholipid, and glycolipid contents of the canola lecithin and its fractions were determined by HPLC-ELSD, which was based on the method used by Sugawara and Miyazawa (1999), with modification. The HPLC system was an 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, mL/mL). 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, mL/mL) 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 lipids in samples.

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, separately. The canola lecithin and its fractions were used as the emulsifiers. A commercial oil-free soy lecithin (Solae, St. Louis, MI) was also evaluated as the reference. The ratio of oil to aqueous phase was 1/9 (g/g), and the total weight of emulsion was 20 g. The weight of emulsifier used was 0.02 g. 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 5 g/kg. The particle size distribution of the emulsions was determined using a High Performance Particle Sizer (HPPS 5001, Malvern Instruments, Malvern, UK). The volume mean diameter, $D_{4,3} = \Sigma d^4/\Sigma d^3$, was calculated. Zeta potential of o/w emulsions was measured using an Acoustic Spectrometer (DT-1200, Dispersion Technology, Bedford Hills, NY). The emulsions were placed in 100 mm graduate cylinders, and covered with parafilm to study emulsion stability at room temperature. The height of cream layer formed at the top of the cylinders were recorded every 20 min for the first 2 h after emulsion preparation. Then data was recorded in 1 h intervals up to the 10th h. Then after, the cream layer height was measured at 24th h, 48th h, 72nd h and 96th h.

2.7. Statistical analysis

All experiments and analytical tests were carried out in duplicate. Means were compared using Fisher's Least Significant Difference method after Analysis of Variance (ANOVA) F-test showed significance. ANOVA was performed using SAS 9.3 (SAS Institute Inc., Cary, NC). All statistical tests were performed at a significance level of 0.05.