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Lipid composition and emulsifying properties of *Camelina sativa* seed lecithin

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ABSTRACT

There is no information on the chemical composition of camelina seed lecithin; therefore, the objective of this study was to investigate the chemical composition and emulsifying properties of lecithin recovered from camelina seed oil by water (WDCL) and enzymatic degumming (EDCL) using phospholipase A_1 (PLA_1). The lecithin obtained by both WDLC and EDLC was rich in phosphatidylinositol (PI), and contents were 37.8 and 25.2 wt%, respectively. Lecithin recovered by enzymatic degumming contained more lysophospholipids compared to water degumming. The saturated fatty acid content of the EDCL was significantly higher than that of the WDCL. Emulsions stabilized using EDCL resulted in the highest stability when deionized water was used as the aqueous phase (original pH); however, at pH = 7.5, emulsions stabilized using EDCL and WDCL were less stable compared to the emulsion stabilized with soy lecithin. Results showed that camelina seed lecithin is a promising alternative PI-rich emulsifier for various food applications.

1. Introduction

Lecithin is a by-product from edible oil processing that has extensive applications in the food, cosmetic and pharmaceutical industries as an emulsifier, solubilizer, anti-spattering agent, cooking spray, crystallization controller and filler, among other functions (Nguyen et al., 2014). Commercial lecithin mainly comprises phospholipids and other vegetable oil fractions including glycolipids (GL), triglycerides (TGA), fatty acids, sterols and carbohydrates dissolved in neutral oils (Van Nieuwenhuyzen & Tomás, 2008). Phospholipids are biologically important compounds, which represent a wide array of species, of which phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA) are the most dominant (Nguyen et al., 2014). The world lecithin market is expected to show a substantial growth and it is estimated that the market could be worth 1.11 billion USD by 2020 (Grand View Research, 2017). This is partly because of the current trend in the food industry where consumers and regulatory bodies are pushing towards replacing synthetic ingredients used for various functionalities, and partly because of reports suggesting the health benefits of phospholipids (Grand View Research, 2017; Marsaoui, Laplante, Raies, & Naghmouchi, 2013). Additionally, increasing demand for convenience foods and nutraceuticals formulated with natural ingredients is also another driver for the surge in demand for lecithin.

The main natural sources of lecithin in the food industry to date are soybeans (Zhu & Damodaran, 2013). By far the most dominant producer and exporter of soybeans is the United States and more than 90% of all the soybeans planted in the United States are genetically modified (USDA, 2016). However, due to increasing skepticism regarding the use of genetically modified foods among consumers, calls for identification of high-quality identity-preserved (IP) lecithin sources have intensified (Van Nieuwenhuyzen, 2014). Moreover, some people are allergic to soybean, and thus having alternative sources of lecithin could be advantageous. Apart from soybean, lecithin is recovered from sunflower kernels, rapeseed, peanut and dairy products (Xie & Dunford, 2016; Zhu & Damodaran, 2013). Because lecithins from different sources have distinct fatty acid and phospholipid compositions, they display different properties. For example, Loncarevic et al. (2016) reported that sunflower lecithin has the lowest viscosity when compared with soy and rapeseed lecithin.

Recent research on the extraction of camelina seed (*Camelina sativa* L. crantz) oil has shown that camelina seed could be a new potential source of lecithin (Belayneh, Wehling, Cahoon, Reddy, and Ciftci, 2017). Camelina seed is an underutilized and ancient oil seed, which has drawn increasing interest in the United States over the past few decades (Belayneh, Wehling, Cahoon, and Ciftci, 2015). Despite the geographical variation in the composition, the seed is rich in protein (40%) and oil (35–40%) (Belayneh et al., 2015). Camelina seed oil

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contains more than 54% polyunsaturated fatty acids (PUFA) and the omega-3 fatty acid α-linolenic acid (C18:3 ω3, ALA) accounts for 30–40% of the total composition. In addition, the oil is rich in tocopherols (760 mg/kg oil) and phytosterols (up to 6500 mg/kg oil), which are health promoting minor lipid compounds (Belayneh et al., 2017). In our previous study, we reported that regardless of the extraction solvent used, the main phospholipids present in camelina seed oil are PI, PC, and PE, respectively (Belayneh et al., 2017).

Lecithin is recovered and purified from gums during refining of vegetable oils to prevent sedimentation and make further processing easier (Loncarevic et al., 2016; Penci, Constenla, & Carelli, 2010). Degumming is usually done either by water or acid, but lecithin used for food application is obtained by water degumming and in some cases by extraction using ethanol (Penci et al., 2010; Xie & Dunford, 2016; Zhu & Damodaran, 2013). Enzymatic degumming, the most recent technique used for degumming, offers a promising approach to obtaining food grade lecithin. Phospholipase A_1 (PLA_1) and phospholipase C (PLC) are the most common enzymes used in the oil and fat industry for degumming. PLA_1 cleaves one fatty acid and results in free fatty acids (FFA) and lysophospholipids as end products. Phospholipase A_2 (PLA_2) is also used when selective cleaving of the fatty acid on the *sn*-2 position is desired (Xie & Dunford, 2016). PLC on the other hand cuts the phospholipid on its phosphate end and leads to diacylglycerol and phosphate ester production (Wang, 2001). Our previous study has shown that camelina seed phospholipid is rich in PI (Belayneh et al., 2017) and the presence of a high amount of PI makes it a prospective emulsifier for water-in-oil emulsions, particularly in the confectionary industry (Hui, 1996) because PI is one of the phospholipid types characterized by its good solidification property, thereby making it a suitable ingredient for the confectionary industry.

There is no reported study on the composition of camelina lecithin and its emulsification properties. There is a critical need for information on the chemical composition of camelina lecithin and its emulsification properties to utilize it in food industry. Therefore, the main objective of this study was to determine the chemical composition and emulsifying properties of lecithin obtained from camelina seed oil by water and enzymatic degumming using PLA_1.

2. Materials and methods

2.1. Materials

Certified organic camelina seed was purchased from a local market in Seattle, WA, USA and the oil was extracted using a screw press (AgoilPress, M70 Oil Press, Eau Clair, Wisconsin, USA). PLA_1 from *Thermomyces lanuginosus* with an activity of more than 10,000 U/g and citric acid were obtained from Sigma Aldrich (St. Louis, MO, USA). Monosodium phosphate and disodium phosphate, sulfuric acid, potassium hydroxide, acetone, petroleum ether and soy lecithin were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Fatty acid standard (triheptadecanoin) was obtained from Nu Check Prep, Inc. (Elysian, MN, USA). Phosphatidylinositol (sodium salt) and lysophosphatidylinositol (LPI, sodium salt) were purchased from Avanti Polar Lipids (Avanti Polar Lipids Inc., Alabaster, AL, USA). All other reagents and solvents were of analytical or chromatographic grade and purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Isolation of camelina seed lecithin

Camelina seed lecithin was isolated using PLA_1 from *Thermomyces lanuginosus* according to Xie and Dunford (2015) with some modification. Cold-pressed camelina seed oil was filtered using #42 Whatman filter paper (GE Healthcare Bio-Sciences, Marlborough, MA, USA). Filtered camelina seed oil (1.5 kg) was heated to 80 °C while mixing using an overhead mixer (RZR1 CAFRAMO, Wiarton, Ontario, Canada). Citric acid (1.95 g, 50% (w/w)) was added into the oil and the mixture was homogenized by a homogenizer (Ultra-TurraxT25, IKA Works, Inc., Wilmington, NC, USA) at 24,000 rpm for 1 min at room temperature (20 °C). Then, the homogenized mixture was stirred for 20 min at 80 °C and 500 rpm using the overhead mixer, and the mixture was cooled to 50 °C in a water bath (HAAKE N3, Thermo Scientific, Waltham, MA, USA). Then, 1.9*g* of 4 N NaOH solution and 2.5% (w/w) enzyme followed by 34.9*g* deionized water was added into the mixture. The whole content was then homogenized at 24,000 rpm for 1 min and stirred at 50 °C and 500 rpm for 6 h using the overhead mixer to allow the reaction to take place. At the end of 6 h, the temperature was increased to 80 °C and the mixture was maintained at that temperature for 0.5 h to deactivate the enzyme. The content was centrifuged using a Beckman J2-21 centrifuge (Beckman Coulter Inc., Brea, CA) at 3000 rpm for 5 min and the gum was washed with cold acetone to separate the lecithin following the AOCS official method Ja 4-46 (AOCS, 2003). The resulting lecithin was flushed with nitrogen and stored at −20 °C.

In parallel, camelina seed oil lecithin was also recovered by water degumming using the same amount of oil. The oil was first heated to 80 °C to facilitate degumming. Deionized water (2.5%, w/w) was added to hydrate the phospholipid. The mixture was stirred at 80 °C and 500 rpm for 1 h. Finally, the mixture was centrifuged at 3000 rpm for 5 min and the lecithin was washed with cold acetone several times (AOCS, 2003).

2.3. Fatty acid analysis

The fatty acid composition of camelina oil lecithin from enzymatic and water degumming was analyzed using a gas chromatograph (GC) equipped with a flame ionization detector (7890A GC systems, Agilent Technologies, Inc., Santa Clara, CA, USA) according to Belayneh et al. (2015) with some modification. Fatty acid methyl esters (FAME) were prepared by weighing 60 mg of lecithin into screw-cap test tubes sealed with Teflon lining. To each tube, 1.5 ml of 2.5% (v/v) sulfuric acid in methanol (containing 0.01% w/v butylated hydroxyl toluene, BHT), and 50 µl of a triheptadecanoin solution (10 mg/ml in toluene) were added. The tubes were capped, and heated at 90 °C for 1 h. Upon cooling, 1 ml of water and 1.5 ml of heptane were added to each tube. Following vortexing and centrifugation at 4500 rpm for 5 min, the heptane layer containing FAME was removed and analyzed by GC. FAME were separated on an Agilent HP-INNOWAX capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature of the GC was held at 90 °C for 1 min and then heated at a rate of 30 °C/min to a final temperature of 235 °C and kept constant for 5 min. Hydrogen was used as a carrier gas. The results were reported as area percentage of the peaks after identifying fatty acids by comparison of their retention times with those of authentic standards.

2.4. Analysis of polar lipids

Polar lipids, including, glycolipids, lysophospholipids and phospholipids, were analyzed using an HPLC (Agilent 1290 Infinity II UPLC, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a Sedex 85 evaporative laser light scattering detector (ELSD) (Sedere, Alfortville Cedex, France) according to Sugawara and Miyazawa (1999) with some modification. The drift tube temperature for the ELSD was set at 80 °C, and the nebulizer gas pressure was 3.2 bar. The flow rate of nitrogen was set at 0.9 standard liter per minute (SLM) and the impactor was ON. Polar fractions were separated on a µPorasil silica column (3.9 mm × 300 mm, 10 µm particle size; Waters Corporation, Milford, MA, USA) using a mobile phase of A: chloroform and B: methanol:water (95:5, v/v). The gradient elution was: 0–15 min, 99–75% A and 1–25% B; 15–20 min, 75–10% A and 25–90% B; 20–35 min, 10% A and 90% B; 35–40 min, 10–99% A and 90–1% B. The oven temperature was set at 35 °C and the flow rate of the mobile phase was 1 ml/min. The lecithin sample was dissolved in chloroform:methanol (1:1, v/v) at a concentration of 20 mg/ml and filtered using a 0.45 µm