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Extraction of phospholipids from a dairy by-product (whey protein phospholipid concentrate) using ethanol

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ABSTRACT

There has been a great interest in the phospholipids (PL) found in dairy products because of their health and functional properties. In this study, a technology that was originally developed for egg yolk PL extraction was applied to whey protein phospholipid concentrate (WPPC). This method successfully precipitated the proteins present in WPPC and extracted the lipids with a renewable alcoholic solvent, ethanol. The effect of ethanol concentration, extraction temperature, and extraction number on the recovery of total lipid, total PL, and individual PL class was evaluated. The optimum processing conditions for a combined 5-stage sequential extraction for producing a PL-enriched lipid fraction were determined to be 70% ethanol at 70°C, and the total lipid recovery, total PL recovery, and PL content achieved were 40.7, 58.1, and 45.8%, respectively. A lipid fraction with high nutritional value (high content of sphingomyelin or phosphatidylserine) can also be obtained by adjusting extraction conditions and collecting specific fractions, although the yield may decrease. Overall, producing a PL-rich lipid fraction from WPPC using ethanol extraction is feasible and scalable, and different processing conditions can be used depending on the type of lipid product desired.
Key words: dairy lipids, ^{31}P nuclear magnetic resonance, sphingomyelin, lecithin

INTRODUCTION

Phospholipids (**PL**), generally referred to as lecithin in the food industry, are a complex class of polar lipids. Phospholipids are amphiphilic because they have a hydrophilic head and hydrophobic fatty acid tail (Donato et al., 2011; Contarini and Povolo, 2013); this gives them excellent emulsification properties and makes them important functional ingredients for the food, pharmaceutical, and cosmetic industries. Other than the technological functionalities such as emulsifying and lubricating, PL have gained considerable interest because of their nutritional value. Although dairy PL represent only 0.5 to 1.0% of total milk lipids, they are of particular interest because of their higher content of sphingomyelin (**SM**; 4.1–29.2% of total PL) and phosphatidylserine (**PS**; 2.0–16.1% of total PL) compared with other lecithin sources such as soybean and egg yolk (Burling and Graverholt, 2008; Contarini and Povolo, 2013). Sphingomyelin is reported to play important roles in cell regulation and is referred to as a tumor suppressor (Rombaut et al., 2006; Dewettinck et al., 2008; Contarini and Povolo, 2013). Cognitive performance improvement, which is of particular significance to Alzheimer's disease treatment, has been attributed to PS (Pepeu et al., 1996; Rombaut and Dewettinck, 2006; Burling and Graverholt, 2008; Dewettinck et al., 2008; Contarini and Povolo, 2013). Other beneficial biological effects such as reduced cholesterol absorption, antioxidant properties, stress and depression tolerance, reduced incidence of cardiovascular disease, and suppression of multiple sclerosis are also associated with dairy PL (Rombaut and Dewettinck, 2006; Dewettinck et al., 2008; Contarini and Povolo, 2013). Unlike soy lecithin, which lacks SM (Nejrup et al., 2017), dairy PL have greater potential to be used in infant formula for mimicking human breast milk, which contains SM and PS (Sala-Vila et al., 2005). Using dairy PL can improve the overall nutritional quality of infant formula as well as the economics of the dairy processing industry.

The majority of the current commercial lecithins are made from soybean and egg yolk. The low PL content in dairy products makes it difficult to extract and concentrate PL on an industrial scale. Commercial dairy PL concentrates are available that are produced by using technologies such as enzymatic hydrolysis of proteins, microfiltration, ultrafiltration, supercritical fluid extraction, or a combination of these technologies (Folch et al., 1957; Astaire et al., 2003; Rombaut et al., 2007; Spence et al., 2009; Costa et al., 2010; Barry et al., 2017). However, these dairy PL concentrates are

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not isolated lipid fractions, and this may limit their application as an industrial lecithin. The commercial dairy product with the highest PL content, Phospholipid Concentrate 700 (Fonterra Co-Operative Group Ltd., Auckland, New Zealand), contains up to 60% (wt/wt) PL (Fong et al., 2013). The processing steps that this product undergoes are an industrial trade secret. This product contains a substantially higher amount of PL than any other product reported in the literature. Previously, the highest PL content reported in a dairy product was 23.7% on a dry basis, concentrated from butter serum using microfiltration (Le et al., 2011). The major limiting factor with microfiltration of buttermilk and butter serum is the retention of casein, which limits how concentrated the PL can become. Developing processing technologies that can be used to produce PL-rich products is a top priority for the dairy industry. Moreover, certain feedstock and technologies used for producing the PL concentrates are not always cost effective, and this leads to low feasibility for commercial-scale production. Therefore, there is a need to develop a more cost-effective, readily scalable extraction method to produce a high-purity dairy PL fraction from an economical source other than buttermilk and butter serum.

Whey protein PL concentrate (**WPPC**) is a byproduct that is microfiltered from cheese whey during the production of whey protein isolate. Whey protein PL concentrate contains 60 to 70% whey protein, and 10 to 30% of the total lipid content is PL (Li et al., 2016). It is a highly underused product and would be a great feedstock for dairy PL concentration. A method that can be used for producing WPPC lipid with high PL content is the simultaneous texturization and extraction of PL (**STEP**) method, which was originally developed to extract PL from liquid egg yolk using ethanol (Wang et al., 2017). The principles in the STEP method may be applied to WPPC for PL concentration, but modifications are needed because the composition of egg yolk and WPPC is very different.

The use of ethanol to extract PL from WPPC was investigated. We hypothesized that (1) lower ethanol concentration can lead to a higher PL content in the final product, whereas higher ethanol concentration can lead to higher recovery yield of total lipids and PL due to the different affinities of lipids to solvent with different polarity, and (2) higher extraction temperatures can result in higher PL content as well as higher recovery of total lipids and PL due to a more complete denaturation of protein and better lipid solubilization in ethanol. To test these hypotheses, different ethanol concentrations and temperature conditions were used for the extraction, and total lipid and PL recovered from WPPC were quantified. Each individual PL class composition was also quantified to evaluate the effect of ethanol concentration and temperature on recovery of each specific class of PL.

MATERIALS AND METHODS

The WPPC used in this study was provided by Bongards' Creameries (Perham, MN). The WPPC was frozen (−20°C) before treatment to guarantee a consistent product quality for each replicate extraction. The extracted lipid was then quantified for PL using ^{31}P nuclear magnetic resonance (**NMR**; Wang et al., 2014). Reagent-grade solvents and other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ) and Sigma-Aldrich (St. Louis, MO).

Extraction of PL from WPPC

The extraction procedure, which has similar extraction principles as the STEP method (Wang et al., 2016, 2017), was performed using WPPC as a feedstock. A laboratory extraction system (Figure 1) designed for the STEP method on egg yolk was adapted to extract total lipid and PL from WPPC. Aqueous ethanol with final concentrations of 70, 80, and 90% was used, and the temperature of the solvent in the solvent reservoir and extraction cylinder was maintained at 60, 70, and 80°C using a hot water jacket heating system (Haake DC 10, Thermo Haake, Karlsruhe, Germany). Liquid WPPC, in the form of a thin stream, was injected using an 18-guage × 1.5-inch PrecisionGlide needle (Becton Dickinson and Co., Franklin Lakes, NJ) into the hot solvent, and the protein in WPPC was denatured and solidified into short strings upon contact with ethanol. The liquid WPPC was heated to 60°C before injection to make the product more flowable, and it was metered using a peristaltic flow pump (UL 3101-1, Wheaton Science Products, Waltham, MA) at a speed of 0.67 g/min through the syringe needle. The needle was manually rotated in a circular motion below the surface of the solvent to obtain uniform protein denaturation and to produce a thin-diameter WPPC string. Liquid WPPC (100 g) was spun into various amounts of ethanol depending on ethanol concentration; 187, 320, and 720 mL of 100% ethanol were used for 70, 80, and 90% ethanol concentration treatment. After protein denaturation, WPPC was immersed in the solvent for 6 min before drainage of the miscella for 1 min. The first extraction was considered complete after the miscella was collected; then, 4 subsequent extractions were carried out on the partially delipidated WPPC using the same conditions, except only 100 mL of the ethanol solvent was used. For the first extraction, the ethanol solvent concentration was 100% so that the concentration of