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# Soybean lecithin composition, fractionation, and functionality

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**Soybean lecithin composition, fractionation, and functionality**

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# TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION ..... 1

Literature Review ..... 1
Thesis Organization ..... 8
References ..... 8

CHAPTER 2. PHOSPHOLIPID CLASS AND FATTY ACID COMPOSITIONS OF MODIFIED SOYBEANS PROCESSED WITH TWO EXTRACTION METHODS ..... 11

Abstract ..... 11
Introduction ..... 12
Materials and Methods ..... 13
Results and Discussion ..... 16
Acknowledgement ..... 21
References ..... 21

CHAPTER 3. SOYBEAN LECITHIN FRACTIONATION AND FUNCTIONALITY ..... 31

Abstract ..... 31
Introduction ..... 32
Materials and Methods ..... 34
Results and Discussion ..... 37
Acknowledgement ..... 45
References ..... 45

CHAPTER 4. GENERAL CONCLUSIONS ..... 57

ACKNOWLEDGMENTS ..... 59

# CHAPTER 1. GENERAL INTRODUCTION

## Literature Review

### Lecithin

Lecithin, according to the International Lecithin and Phospholipids Society (ILPS), is a mixture of glycerophospholipids obtained from animal, vegetable or microbial sources, containing a variety of substances; such as sphingosylphospholipids, triacylglycerols, fatty acids and glycolipids (1). Lecithin could be used as an emulsifier to stabilize dispersions or to coat surfaces, which is exploited in the manufacture of food, the production of cosmetics, the formation of liposomes employed as drug carriers, the stabilization of fat emulsions for intravenous administration, and for many industrial applications (2). Egg yolk is the main source of animal lecithin, and soybeans is the predominant plant source of lecithin (3). The main difference between soybean lecithin and egg lecithin is that the former has a higher unsaturated fatty acid and less choline content and has no cholesterol. Egg lecithin as a commercial ingredient, with the exception of some medical feeding programs, is too expensive for routine use in food (4). Whereas soybean lecithin, based on its wide spread availability, is extensively used in food, pharmaceutical and cosmetic industries.

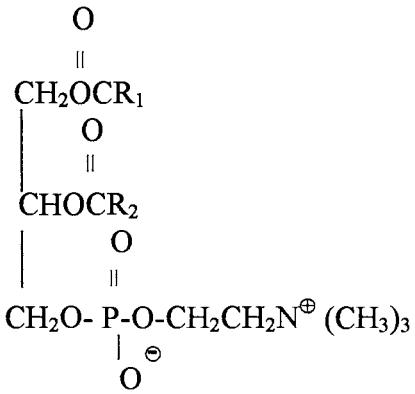
### Nutrition and Health of Lecithin

Lecithin seems to have numerous health benefits, including aiding in the reproductive process and fetal development, liver health, heart health, improved memory, and improved physical performance (5). Many of the positive effects of lecithin consumption are based on the fact that lecithin is a major source of choline. Choline aids in the digestion of fats and in

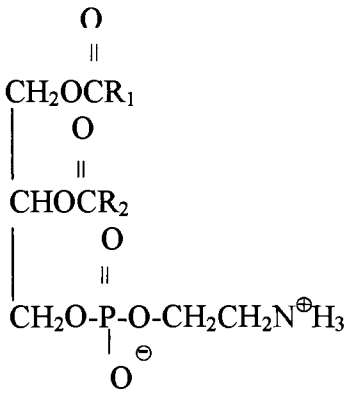
reducing high blood cholesterol concentrations. High blood cholesterol levels lead to congestion of blood vessels and therefore is often an important risk factor in heart attacks and strokes. Choline also is a part of the neurotransmitter acetylcholine. A sufficient intake of choline, primarily via the consumption of lecithin, is believed to have a positive effect on some mental functions, especially those related to memory (6).

**Soybean Lecithin Composition**

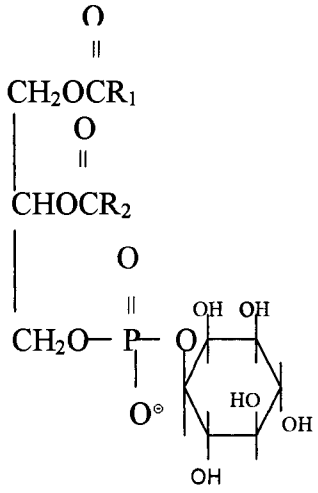
Crude soybean lecithin is obtained as a by-product of soybean oil processing. By adding 1-3% water to crude soy oil and with gently stirring, phospholipids (PLs) will hydrate and become insoluble in soybean oil. Thus gum can be obtained by centrifuging the oil and crude lecithin obtained by drying. Soybean lecithin is mainly composed of phospholipids, which usually have two hydrophobic fatty acyl chains and a polar hydrophilic head group. These unique chemical and physicochemical properties determine its role as structural components of biological membranes as well as its usefulness in numerous practical applications. The main PLs in soybean lecithin are phosphatidylcholine (PC), 55.3%; phosphatidylethanolamine (PE), 26.3%; and phosphatidylinositol (PI), 18.4% (7). The structures of these PLs are as follows:



Phosphatidylcholine



Phosphatidylethanolamine



Phosphatidylinositol

Due to the different head groups, the three PLs have different functional characteristics. Thus, functional properties of the lecithin product may be different if the relative proportion of the PLs classes is different (8). The modification of fatty acid composition in PLs may also affect the functional properties of lecithin such as emulsification property and oxidative stability (8). So, it is important to determine the fatty acid composition as well as the PLs class composition and to study how these compositional changes affect PL functional properties.

Soybean oil production in commercial facilities usually employs solvent extraction (SE) methods, which use organic solvent such as hexanes, to extract oil from soybean flakes. With this method, a greater proportion of oil could be recovered and less heat treatment needs to be applied to the seed prior to oil extraction. However, there are also several disadvantages of this method such as intensive capital in establishing plants and infrastructure, high energy consumption, and safety and environmental concerns over flammable solvents. Recently, farmers in North America are increasingly building extrusion-expelling (E-E) facilities to add value to their soybean products rather than selling soybeans to large processing plants. The mechanical processing uses a dry autogenous extruder in which the pressure and heat generated disrupt the cellular structure of the seed and a screw press is then used to press out the oil (9). Compared to SE plants, E-E mills are much less expensive to construct. More importantly, the oil from E-E processing is not treated with organic solvent, so the E-E oil could be regarded as "natural food", which is appealing to many consumers. Different oil extraction methods could affect the minor component profile of the oil. For example, E-E crude oil contains low levels of phosphatides and free fatty acids (9-12), and extruder-treated soy flakes resulted in soybean oil (solvent extracted) with different PLs composition (13). In the present research, the effect of oil extraction method on PL class and fatty acid composition of lecithin will be examined.

Soybean oil belongs to linolenic acid group of edible oils; it contains a relatively high amount of linolenic acid that is highly prone to oxidation. The lipoxygenase iso-enzymes present in soybeans catalyze the oil oxidation once seeds are broken and may result in beany flavor. So, it may be desirable to genetically modify soybean to produce an oil with improved oxidative stability. Soybeans with increased oleate and decreased linolenate percentages and

with lipoxygenases removed are developed. In addition, some modification could be made to increase the nutritional quality of soybean oil, such as lowering the saturated acid content. Such modifications change the fatty acid profile of soybean oil, which could result in altered fatty acid profiles of PLs (7) and thus could affect the functional properties of lecithin (8). In this research, the fatty acid composition of PLs classes of the modified soybeans will also be determined.

**Soybean Lecithin Fractionation**

Crude soybean lecithin usually contains 18% PC, 14% PE, 9% PI, 5% phosphatidic acid (PA), 2% of minor PLs, 11% glycolipids, 5% complexed sugars, and 37% of neutral oil (2). Deoiling of crude lecithin is considered very important in making high-purity lecithin products. Acetone is currently used in industry for separating of neutral oil and PLs, based on the fact that neutral oil is soluble in acetone while PLs are insoluble. Deoiled lecithin typically contains 23% PC, 20% PE, 14% PI, 8% PA, 8% minor PLs, 15% glycolipids, 8% complexed sugars, and only 3% neutral lipids (5).

It is also desirable to fractionate deoiled lecithin into PC-enriched and PI-enriched fractions for certain applications, because these two fractions have different functionalities. For example, PC has a high hydrophilic-lipophilic balance (HLB) value, and thus can be used to promote and stabilize o/w emulsions; PI has a low HLB value, thus is effective in promoting and stabilizing w/o emulsions (14). The main commercially utilized processes for such fractionation uses short-chain alcohols, such as methanol, ethanol, and isopropanol, because PC is relatively more soluble in these alcohols, PI is relatively insoluble in these solvents, and PE is partially soluble (15).

Currently, crude lecithin is first deoiled with acetone and then fractionated with alcohol. It may be possible that the oil remaining in the lecithin acts as a co-solvent during alcohol fractionation. If so, the crude lecithin could be more efficiently fractionated without acetone deoiling. Oil would be remaining in PI fraction because this fraction is considered more non-polar compared with the PC fraction, so only this fraction needs to be deoiled with acetone. In this way, the yield of the two parts may be increased and the fractionation efficiency may be improved. Furthermore, acetone that is considered flammable and toxic could be avoided for producing PC fraction; thus, this fraction could also maintain its "natural" characteristic. This may provide additional opportunities for small soybean processors who employ E-E processing to add value to their products. Fractionation of crude lecithin without dangerous solvents presents possibility of making such products. In this research, we fractionate crude lecithin and evaluate its production, analyze PLs class composition of the two fractions, and compare them with those produced from deoiled lecithin.

**Functionalities of Fractionated Soybean Lecithins**

Soybean lecithin is widely used in food, pharmaceutical, and cosmetic industries based on its emulsification, blending, and instantizing functionalities (16). Among these, emulsification is the most widely utilized. It was reported that about 80% of the soy lecithin produced is sold as food emulsifiers and 20% is used in a variety of industrial applications (16). This is also true for the fractionated lecithins, i.e., PC and PI fractions, although they are used in promoting and stabilizing different types of emulsions. Some properties related to their emulsification characteristic, such as surface tension reduction of aqueous system,

critical micelle concentration (CMC), emulsion stability, and oxidative stability of the fractionated lecithins, will be tested in this research.

Emulsions are thermodynamically unstable systems. The addition of stabilizing additives is one of the tools for creating stable emulsions (17). Lecithin is this type of additive, which adsorbs to an interface to decrease the interfacial tension. According to Heimenz, $\Delta G=\gamma_i \Delta A$, where $\Delta G$ is the free energy required to increase the contact area between the two immiscible liquids by $\Delta A$, and $\gamma_i$ is the interfacial tension. Therefore, after adding surface-active agent to reduce interfacial tension, same energy input will result into bigger $\Delta A$, thus smaller dispersed particle size (18). Based on Stokes' law, smaller particle size results in lower creaming velocity and therefore a more stable emulsion (19). So, the smaller the interfacial tension, the more stable emulsion could created.

CMC is another index of emulsification. Below the CMC, surfactant molecules are dispersed predominantly as monomers, which has no effect on creating an emulsion; but, above this concentration, the surfactant molecules form micelles, hence, surface tension becomes essentially independent of concentration (20, 21). Therefore, the lower the CMC, i.e., the smaller the amount of emulsifier required to form micelles and thus to perform its emulsifying function, the more effective the emulsifier would be.

Emulsion stability refers to the ability of an emulsion to resist changes in its physical properties over time; the more stable the emulsion, the more slowly the phases separate (19). According to Stokes' law, emulsion stability is determined by the diameter of the dispersed droplets, the density difference between the continuous and discontinuous phases, and the viscosity of continuous phase. In the case of all the conditions of continuous and discontinuous phases are same, the emulsion stability would be determined only by the

emulsifiers added, because it is the only factor that could affect diameter of droplet as explained above.

Oxidative stability is one concern for use of lecithin as additives in food, pharmaceutics, and cosmetics because it is a class of polar lipids with higher proportion of unsaturated fatty acid than that of the source oil (15). In addition, lecithin stays at the surface or interface when being used as an emulsifier, which gives it greater chance of exposure to air or other conditions. Thus, it has a high risk of being oxidized. The oxidation of unsaturated fatty acids in PLs is similar to that of free acids or methyl esters. Primary products are diene hydroperoxides formed in a free radical process (22). The final products, off-flavor compounds, such as aldehydes and ketones, will give the entire product an unpleasant flavor. So, it is important to know the oxidative stability of lecithin before using it in many applications.

## Thesis Organization

This thesis contains a general introduction, followed by two research papers and a general conclusion. The papers are in the required corresponding journal formats.

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# CHAPTER 2. PHOSPHOLIPID CLASS AND FATTY ACID COMPOSITIONS OF MODIFIED SOYBEANS PROCESSED WITH TWO EXTRACTION METHODS

A paper to be submitted to *the Journal of American Oil Chemists' Society*

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## Abstract

Soybean lecithin is used as an emulsifier in the food, cosmetic, and pharmaceutical industries. The proportion of individual phospholipids (PLs) and their fatty acid (FA) composition may affect the functional properties of lecithin. In this research, lecithins recovered from four modified soybeans and one commodity soybean were processed by extrusion-expelling (E-E) and conventional solvent extraction (SE), and were analyzed for PLs class proportion and fatty acid composition. High-performance liquid chromatography (HPLC) with an evaporative light scattering detector analysis demonstrated that the phosphatidylcholine percentage in E-E lecithin was higher than that in SE lecithin, whereas the phosphatidylethanolamine content was lower. Gas chromatography (GC) analysis showed that FA composition of the PLs varied with soybean type. Oil extraction method did not significantly affect FA composition. Critical micelle concentration determined with a tensiometer showed differences among the lecithins.

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**Key words:** Critical micelle concentration, extrusion-expelling, fatty acid composition, genetically modified soybeans, phospholipids, solvent extraction, soy lecithin.

## Introduction

Soy lecithin, a general term for total soybean phospholipids (PLs), is widely used in food, cosmetic, and pharmaceutical industries as an emulsifier, lubricant, and release agent (1). It is a by-product of soybean oil processing. PLs are hydrated and settled as gum from oil during degumming. The physical stability of the oil is improved and lecithin is produced after the gum dries. PLs in soy lecithin are approximately 55.3% phosphatidylcholine (PC), 26.3% phosphatidylethanolamine (PE), and 18.4% phosphatidylinositol (PI) (2). Each of these phosphatides has two fatty acyl chains and a polar head group, which give the surfactant characteristics to lecithin. Due to the differences in their head groups, the three PLs have different functional characteristics. The functional properties of the lecithin product may vary if the relative proportions of the PL classes vary (3).

Soybean oil can be extracted by two different methods: mechanical pressing, such as extrusion-expelling (E-E), and solvent extraction (SE). SE processing uses hexane and is a common practice for conventional oil processing plants (typically 3,000 t/d) (4). E-E technology uses a dry autogenous extruder, which generates pressure and heat to disrupt the cellular structure of the seed. A screw press is then used to press the oil out. E-E plants are inexpensive to construct and operate, and value-added soybean products may be produced. Farmers in North America are increasingly building E-E facilities (74 plants in the Midwest soybean-producing area as of spring 1999) (4). Different oil extraction methods affect the profile of minor components of oil; for example, E-E crude oil contains low levels of PLs

and free fatty acids (4-7). It has been reported that the PL profile of lecithin could be changed by different oil extraction methods; for example, lecithin from the expander/solvent extraction process contains more PC and less PE than does SE lecithin (8).

Soybeans can be genetically modified either to improve oxidative stability. For example, high-oleate (HO), low-linolenate (LLL), and lipoxygenase-free (LOX) soybeans. Soybeans may also be modified to obtain a highly nutritional oil such as the low-saturates (LS) soybean. Oilseed modification changes the fatty acid (FA) profiles of the PLs (3), and this change may in turn affect the functional properties of the lecithin, such as emulsification and oxidative stability (2). So, it is important to determine the PLs class and fatty acid compositions of the PLs of soy lecithin.

The objective of the present research was to determine whether and how soybean lecithins from the two oil processing methods, as well as from genetically modified soybean seeds differed regarding PLs class and FA compositions. Lecithins from four types of genetically modified soybeans and one from commodity soybean processed with both E-E and SE processing were analyzed for their PLs class profile and the FA compositions in each PL class. The critical micelle concentration (CMC) also was determined to examine the functionality of soy lecithin. CMC is an emulsifier index, with a lower CMC indicating better emulsification capability (9).

## Materials and Methods

*Soybean seed sources.* Commodity soybeans (CS) were obtained from West Central Cooperative (Ralston, IA) and were used for comparing the PLs class proportions and FA profile of individual PLs with other genetically modified soybeans. Three soybean varieties

with modified fatty acid compositions were obtained from Optimum Quality Grains (Des Moines, IA): a HO line, A2333HO, containing 79.2% oleate; a LS line, P92B72, containing 8.4% total saturated fatty acids; and a LLL line, P9322, containing 3.1% linolenate. A LOX line, IA2027, provided by the Committee for Agricultural Development, Iowa State University (Ames, IA), was also used. FA composition of these seeds is illustrated in Figure 1.

*Soybean processing.*

*(1) E-E processing.* Five types of soybeans (20 bu each) were processed at a commercial E-E plant (Iowa Soy Specialties, Vinton, IA) by using an Insta-Pro extruder (Model 2500) and a screw press (Model 1000). The seeds were cracked with a roller mill and dehulled by aspirating, and the meats were extruded and expelled. The meals and oils were collected in an identity-preserving fashion after the residuals from the previous seed lots were flushed and steady-state operating parameters were achieved.

*(2) SE processing:* A pilot-plant-scale solvent extractor simulator (French Oil Mill Machinery Co., Piqua, OH) was used to extract one bushel of dehulled and flaked soybeans. Five stages of hexane extraction at 60°C were used. The majority of the hexane was then evaporated with an evaporator/stripper and the residual hexane was removed with a laboratory rotary evaporator at 65°C.

*Gum collection.* Crude oils extracted by E-E and SE methods from the five types of soybean seeds were filtered to remove meal fines; then, 3% water was gently stirred into the oil to hydrate the PLs at 60°C for about 1 h. Then, the mixture was centrifuged, oil and gum were separated, and the gum was collected.

*Lecithin sample preparation.* Crude gum obtained from degumming contains a high proportion of neutral oil that may affect the analysis of PL. PLs are insoluble in acetone whereas neutral oils are soluble (10). Acetone treatment according to AOCS Official Methods Ja 4-46, Procedures 1-5, was used to de-oil the crude lecithins (11). Deoiled lecithin was vacuum oven-dried at 60°C for 24 h to remove moisture as well as residual acetone.

*Fatty acid composition analysis.* Lecithin samples dissolved in chloroform were streaked onto 20 x 20 cm, 500-um Adsorbsil preparative plates (Alltech Associates, Inc, Deerfield, IL). The plates were developed with chloroform/methanol/acetic acid/water (100:50:5:2). Bands were visualized by spraying with 0.1% 2', 7'-dichlorofluorescein in methanol and viewing under UV light. Different PLs were collected in separate vials. Fatty acid methyl esters (FAME) were obtained by direct transesterification of the silica bands with 1 M sodium methoxide in methanol; FAMEs were analyzed by gas chromatography (GC). The GC used was Hewlett-Packard (HP) (Avondale, PA) 5890A equipped with a flame ionization detector and capillary fused column (15 m length, 0.25 mm id, and 0.20 um film thickness) from Supelco (Bellefonte, PA). Oven temperature was 200°C; inlet and detector temperatures were 250°C. Split ratio was 10:1.

*PL class separation and quantification with HPLC.* A Beckman Coulter (Fullerton, CA) HPLC system with auto sampler 508, solvent delivery module 126, silica column (250 mm length, 2.1 mm id, from Alltech), and evaporative light scattering detector (ELSD 2000, from Alltech) was used for the PL class composition analysis. Two solvent mixtures were used: "A" was chloroform/methanol/ammonium hydroxide (80:19.5:0.5, vol/vol), "B" was chloroform/methanol/water/ammonium hydroxide (60:34:5.5:0.5, vol/vol). Flow rate was 0.3 mL/min. Nitrogen gas at 1.6 L/min flow rate was used to evaporate the solvent in the heated

chamber at 50°C. The gradient program of these two mobile phases used in this research is shown in Table 1.

*Standard calibration curves for each individual PL class.* PC, PE, and PI standards with purity greater than 99% (Avanti Polar Lipids, Inc., Alabaster, AL) were dissolved in chloroform at different concentrations and analyzed with the HPLC/ELSD 2000 under the above-mentioned conditions. Relationships between peak area and sample injection quantity were plotted to obtain a standard calibration curve for each of the PL classes.

*Statistical data analysis.* Data were analyzed with the General Linear Model of SAS program (12). A factorial experimental design was used to examine the effects of soybean seed type (five seeds) and extraction method (two methods) on FA and PLs class compositions. The least significant differences (LSD) at $P = 0.05$ were calculated to compare treatment differences. Each processing and analysis method was duplicated.

*CMC determination for lecithins.* Lecithins were dispersed in water at high concentrations, and then diluted with water to lower the concentrations. Surface tensions at each concentration were tested with a FACE Automatic Surface Tensiometer (CBVP-Z, Tantec Inc., Schaumburg, IL). Surface tensions were plotted against concentrations. The declining section and the horizontal section of the curve were analyzed separately to obtain the linear trend of each. The intersection of the two lines where the surface tension started to become constant with increased concentration was considered to be the CMC of the lecithin.

## Results and Discussion

*FA composition.* Statistical analysis demonstrated that there was no interaction between extraction method and soybean type. Generally, extraction method did not significantly affect

the FA composition of the PLs classes (Table 2). The average percentages of FA in individual PLs of E-E and SE lecithins of the five soybean seeds are presented in Figure 1. Fatty acid compositions of triacylglycerol (TAG) from the five soybean types were also included to compare the difference in composition between TAG and PLs.

*Soybean type caused significant differences in FA composition of all the PLs classes.* HO PL had the most unusual FA profile. It contained only about half the palmitate in all the PLs classes as compared with the other four soybean types, which had similar palmitate contents of both TAG and PLs. HO also contained the least stearate in both TAG and PL, but the difference was not as significant as for palmitate content. The oleate content of HO was considerably different from the other four soybean types. HO contained about 80% oleate in TAG and 60% in PL classes, while the others only contained about 10-30%. The higher oleate content in HO was balanced with the lower content of linoleate. All the other four types of soybeans contained about 50-70% of linoleate, but HO only contained about 10-30% in both TAG and PL classes. LS was expected to contain less saturated FA in oil, but our results did not show this trend in PLs. LS had less palmitate in TAG, but its level was similar in PL to that of the other four. As for stearate, its level was slightly less in TAG than in PL. It seems that the genetic modification of LS did not result in significantly lower contents of saturated FAs in PLs. In general, the FA profiles of LS in the three PLs were similar to the other soybean types except for HO. LLL contained the least amount of linolenate in both TAG and PL, with a decrease of about 5% compared with CS. LOX had no significant difference on the contents of all the FAs compared with CS.

FA profiles of TAG and PLs were also different. The palmitate and stearate contents of TAG were similar to those of PC and PE, but different from those in PI. PI contained

much more of the two saturated FAs than did TAG, PC, and PE. The oleate content of TAG was about 20% higher than that of all three PLs, while the linoleate content of TAG was lower compared with that of the PLs. The linolenate content of TAG and PLs were not significantly different.

Comparing the three PLs, PI contained more saturated and less unsaturated FAs than did PC and PE. This characteristic of PI and its unique head group structure may give PI different properties from PC and PE, such as different solubility in ethanol, which is the basis of fractionating of PC and PI.

*HPLC quantification of PLs classes.* PC, PE, and PI standards with different concentrations were separately injected into HPLC. The standard calibration equations for the three PLs are as follows (X represents micrograms of PLs and Y represents peak area):

PC: $Y = 10^6X + 680{,}998$ $(R^2 = 0.9912)$

PE: $Y = 909{,}079X^{1.228}$ $(R^2 = 0.9985)$

PI: $Y = 452{,}779X^{1.339}$ $(R^2 = 0.9995)$

Melton (13) reported linear calibration curves for PE (10-150 ug), PC (10-250 ug), and PI (10-75 ug); Balazs et al. (14) reported linear calibration curves for all three PLs with 2-8 ug PLs injection, whereas Christie claimed non-linear relationships for all of them with the injection to be 1-5 ug (15). In our injection range, a linear result was obtained for PC (1.25-25 ug) and non-linear results for PE (0.5-25 ug) and PI (0.25-10 ug). It was obvious that the injection ranges of the above were quite different. It was reported that linear response of the evaporative light scattering detector decreased drastically below 10 ug PLs (13). It seems that injection of PLs at higher amounts results in a linear response in the detector. This is in agreement with our results, as shown above, that the PC calibration curve, which started

from a higher injection amount than PE and PI, resulted in a linear relationship between injection amount and detection response while PE and PI did not. We used the higher starting injection amount of standard PC because the PC contents in our samples were higher than PE and PI, so the lower range of injection was not necessary. It was also reported that the linear range depended on the specific commercial model of the detector and it largely depended on the nebulizer design (15, 16). In our study, PL classes were well-separated, and a smooth baseline and good reproducibility were obtained. Retention times for the three PLs became closer to each other after continuous analysis because of the water-containing mobile phase. Balazs et al. (14) reported that equilibrating silica columns was difficult when water was present in the mobile phase.

Contents of each individual PLs class were quantified by using the above standard curves. Total amounts of PLs in the ten samples are shown in Table 3. The purities of the lecithins were still very low, although they were de-oiled with acetone. This corresponds with our observation that the de-oiled lecithins appeared very oily. There still was a considerable amount of oil remaining in the lecithin. To obtain purer lecithin, multiple acetone precipitations may be needed. Table 3 also shows that LS lecithins from E-E and SE processing were significantly different. This may be the result of experimental errors, such as non-uniform sampling or inconsistent deoiling.

The relative proportions of PL classes for the five soybean types and two extraction methods are presented in Figure 2. Extraction method significantly affected PL composition and there was significant interaction between extraction method and soybean type. E-E lecithin contained significantly more PC and less PE than did SE lecithin. E-E processing resulted in a superior PL profile of lecithin because the lecithins enriched in PC are

considered better hydrophilic emulsifiers in cosmetic and pharmaceutical products (17). In addition, E-E oil is processed mechanically without any organic solvent treatment, so it is more appealing to consumers who prefer natural foods or goods.

Zhang et al. (8) reported that expander-processed lecithin contained 39.8% PC and 12.4% PE (based on acetone-insoluble (AI)), whereas non-expander treated lecithin contained 34.2% PC and 18.1% PE, and almost the same percentage of PI. This is in agreement with our results in that mechanically processed lecithin contained more PC and less PE than that of non-mechanically processed soybeans.

Soybean seed type significantly affected the PL composition (Table 4). PC and PE contents of CS and LOX lecithin obtained from both E-E and SE extractions were similar between the two soybean types but they were significantly different from those of the other three soybeans. It seems that the genetic modification of LOX did not affect the relative PC and PE contents, probably because the LOX seeds were only modified to remove the lipoxygenases. PC contents of LLL, HO, and LS from both E-E and SE lecithins were all higher than those of E-E and SE lecithins from CS, whereas PE contents were all lower. It seems that genetic modification of these seeds could not only improve the oxidative stability of their oils, but also increase the PC content in their lecithins, adding value to their lecithin products.

*CMC determination of the lecithins.* PL molecules contain both a lipophilic fatty acyl group and a hydrophilic head group, and this feature gives the PL surface tension reduction capability, which could be quantified by CMC. Above the CMC, the thermodynamic activity of emulsifier does not increase with the addition of more emulsifier (9); in other words, the surface tension is not reduced further with the addition of more emulsifier. A smaller CMC

suggests better emulsification capability. The CMCs of the ten lecithins were determined and presented in Table 5. There were no significant correlations between CMC and composition of individual PL class and FA ($P>0.05$). Because of the high impurity content of our samples and the number of sample types, it was difficult to correlate the CMC with single factor directly. However, there are certain trends that may be obtained from plotting CMC with PL class composition. CMC decreased as PC and PI proportions increased, with the slope of PI being greater than of PC. This result may indicate that a change in PI proportions could make more drastic changes in CMC than a change in PC.

## Acknowledgement

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**TABLE 1.**
**Gradient Program of Mobile Phase in HPLC Analysis of PLs**

| Times (minutes) | Solvent A * (%) | Solvent B** (%) |
|---|---|---|
| 0 | 100 | 0 |
| 5 | 45 | 55 |
| 10 | 30 | 70 |
| 20 | 30 | 70 |
| 25 | 0 | 100 |
| 30 | 0 | 100 |
| 35 | 50 | 50 |
| 40 | 100 | 0 |

* Solvent A: chloroform/methanol/ammonium hydroxide = 80: 19.5: 0.5 vol/vol.
** Solvent B: chloroform/methanol/water/ammonium hydroxide (30%) = 60: 34: 5.5: 0.5 vol/vol.

**TABLE 2.**
**$LSD_{0.05}$ and *P* Values for the Effects of Extraction Method and Soybean Type on FA Profile (number of replication = 2)**

| PL class | Variable | | Fatty Acid | | | | |
|---|---|---|---|---|---|---|---|
| | | | Palmitate | Stearate | Oleate | Linoleate | Linolenate |
| PC | Processing Method* | $LSD_{0.05}$ | 2.2 | 0.7 | 3.61 | 3.9 | 0.75 |
| | | *P* value | 0.7517 | 0.0023 | 0.4219 | 0.3265 | 0.0012 |
| | Soybean Type** | $LSD_{0.05}$ | 1.39 | 0.44 | 2.29 | 2.46 | 0.48 |
| | | *P* value | <0.0001 | 0.0002 | <0.0001 | <0.0001 | <0.0001 |
| PE | Processing Method | $LSD_{0.05}$ | 1.37 | 0.58 | 1.5 | 1.72 | 0.06 |
| | | *P* value | 0.3702 | 0.0523 | 0.1921 | 0.14 | 0.0102 |
| | Soybean Type | $LSD_{0.05}$ | 2.16 | 0.92 | 2.37 | 2.72 | 0.98 |
| | | *P* value | <0.0001 | 0.0017 | <0.0001 | <0.0001 | <0.0001 |
| PI | Processing Method | $LSD_{0.05}$ | 1.83 | 1.08 | 1.6 | 1.62 | 0.79 |
| | | *P* value | 0.1557 | 0.015 | 0.9545 | 0.9435 | 0.8397 |
| | Soybean Type | $LSD_{0.05}$ | 2.87 | 1.71 | 2.53 | 2.57 | 1.26 |
| | | *P* value | <0.0001 | 0.0334 | <0.0001 | <0.0001 | 0.0003 |

* Extraction methods refer to soybean oil extraction methods: E-E and SE.
** Soybean types refer to the five genetically modified soybeans: CS, LLL, LOX, HO, and LS. CS is commodity soybean, LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, and LS is low-saturates.

**TABLE 3.**
**PL Percentages of Acetone Deoiled Soybean Lecithins Recovered from E-E and SE Processing**

| | CS* | LLL | LOX | HO | LS |
|---|---|---|---|---|---|
| E-E | 24.69 | 17.01 | 21.04 | 22.68 | 40.84 |
| SE | 29.26 | 15.24 | 22.97 | 22.77 | 17.51 |

* CS, LLL, LOX, HO, and LS are types of soybean seeds. CS is commodity soybean, LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, and LS is low-saturated.

**TABLE 4.**
**$LSD_{0.05}$ and *P* Values for the Effects of Extraction Method and Soybean Type on PL Class Composition (number of replication = 2)**

| Variable | | Phospholipids | | |
|---|---|---|---|---|
| | | PC | PE | PI |
| Processing Method* | $LSD_{0.05}$ | 2.2 | 0.7 | 3.61 |
| | *P* value | <0.0001 | <0.0001 | 0.0028 |
| Soybean Seed Type** | $LSD_{0.05}$ | 1.39 | 0.44 | 2.29 |
| | *P* value | <0.0001 | <0.0001 | 0.0735 |

* Extraction methods refer to soybean oil extraction methods: E-E and SE.
** Soybean seed types refer to the five genetically modified soybean seeds: CS, LLL, LOX, HO, and LS. CS is commodity soybean, LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, and LS is low-saturated.

**TABLE 5.**
**CMC (mg/ml) of Lecithins from Five Types of Soybean Processed with E-E and SE**

| Process | Soybean Type | | | | |
|---|---|---|---|---|---|
| | CS* | LLL | LOX | HO | LS |
| E-E | 1.34 | 1.63 | 1.75 | 2.5 | 4.13 |
| SE | 4.04 | 1.87 | 5.45 | 1.18 | 3.63 |

* CS, LLL, LOX, HO, and LS are types of soybean seeds. CS is commodity soybean LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, and LS is low-saturated.

**Figure captions:**

**Fig. 1**. Fatty acid percentages of TAG and PL classes in 5 types of soybean: CS, LLL, LOX, HO, and LS. CS is commodity soybean, LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, LS is low-saturated.

**Fig. 2.** PLs percentages of lecithins from E-E and SE processing of five types of soybean: CS, LLL, LOX, HO, and LS. CS is commodity soybean, LLL is low-linolenate, LOX is lipoxygenase-free, HO is high-oleate, LS is low-saturated.

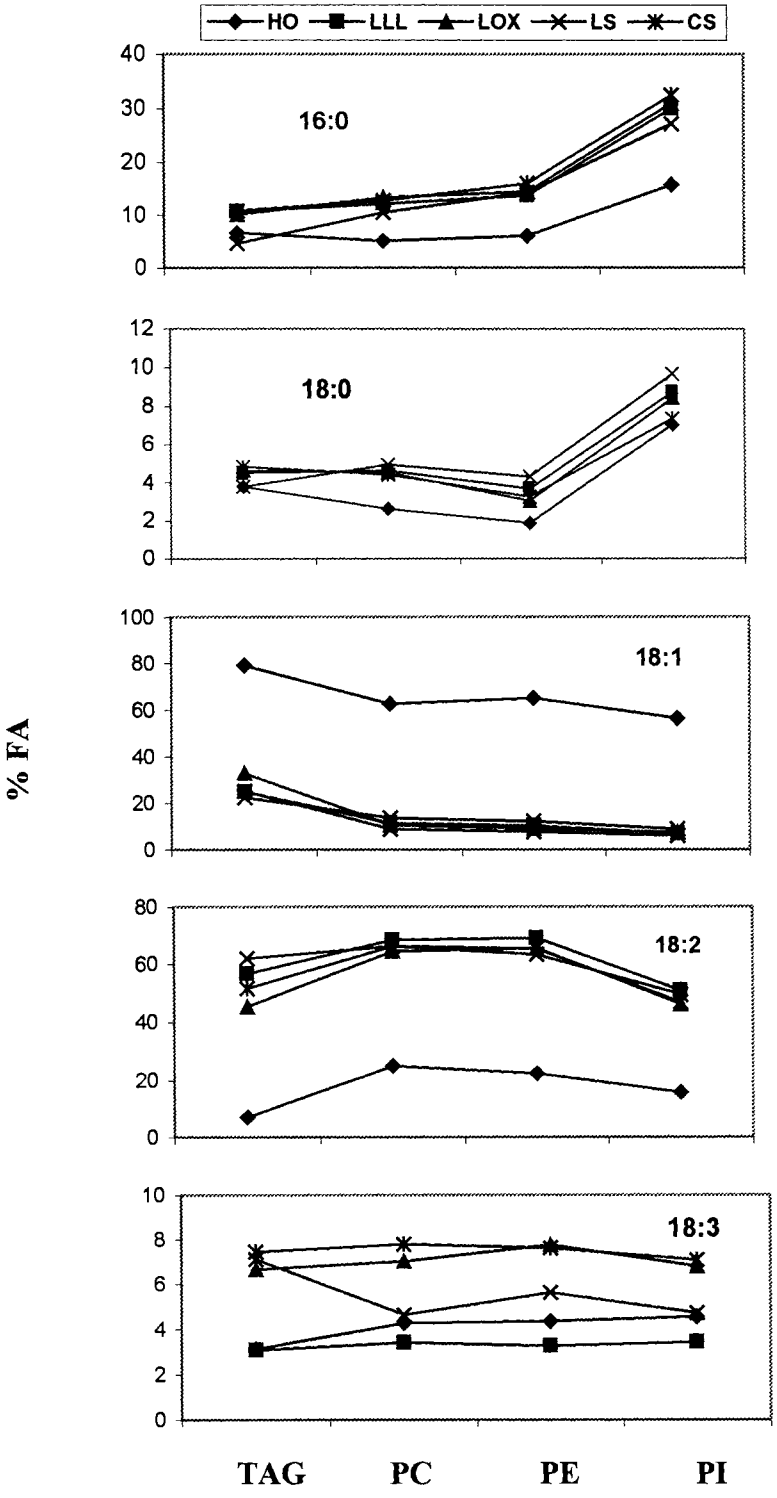
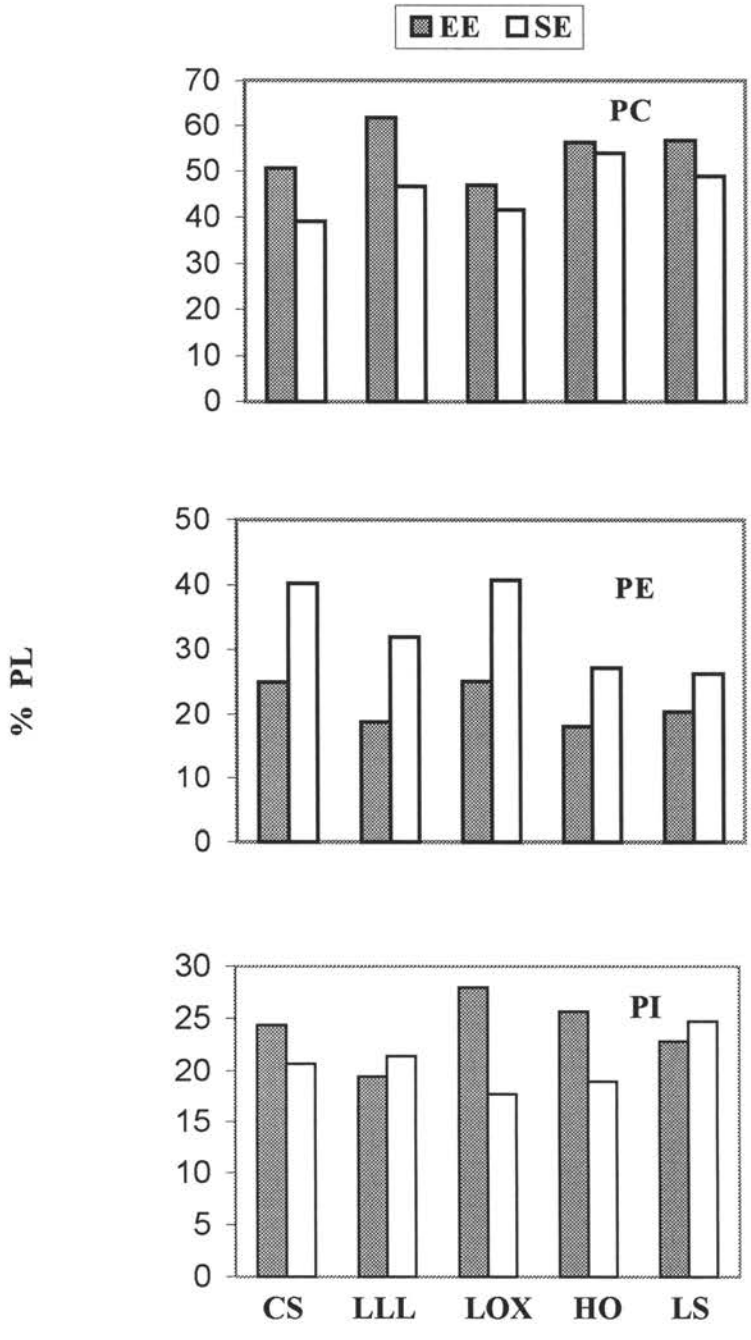



**Figure 1**


EE
SE
PC
PE
PI
% PL
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20
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5
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CS
LLL
LOX
HO
LS

**Figure 2**

# CHAPTER 3. SOYBEAN LECITHIN FRACTIONATION AND FUNCTIONALITY

A paper to be submitted to *the Journal of American Oil Chemists' Society*

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## Abstract

Soybean lecithin primarily contains phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Ethanol was used to fractionate PC and PI, which have different solubilities in this solvent. Various concentrations of ethanol (90, 95, and 100%) and ethanol to gum ratios (0.5, 1.0, 1.5, 2.0, and 2.5) were used. Ethanol concentration significantly influenced the yield of the PC-enriched fraction and the PC and PI fractionation: the highest ethanol concentration resulted in the highest yield of PC fraction, the most PC in the PC fraction, and the most PI in the PI fraction. The ratio of ethanol to gum significantly affected the yield of the PC-enriched fraction, but did not affect the relative PL composition of PC-enriched fraction. Ethanol (90%) at 3 solvent: gum ratio was used for large scale fractionation. Fractionation gave a PC-enriched fraction containing 73% PC, 24% PE, and 3% of PI based on total PLs content, whereas the PI fraction contained 26% PC, 35% PE, and 39% PI. The PI-enriched fraction had much lower critical micelle concentration than did the PC- enriched fraction, which suggested the PI-enriched fraction has a higher surface tension reduction capability. PI-enriched fraction had better emulsion stability than did the PC fraction in both w/o and o/w emulsions. These PLs were very stable to lipid oxidation.

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**Key words:** Critical micelle concentration, ethanol fractionation, emulsion stability, oxidative stability, soybean lecithin functionality.

## Introduction

Lecithin is widely used in food, pharmaceutical, and cosmetic industries. The percentage distribution of lecithin products among the various sectors are: margarine, 25-30%; baking/chocolate and ice cream, 25-30%; technical products, 10-20%; cosmetics, 3-5%; and pharmaceuticals, 3% (1). Soybeans is the predominant plant source of lecithin because of its abundant availability as well as the outstanding functionalities of its lecithin. Crude soybean lecithin is obtained as a by-product of soybean oil processing (degumming). It typically contains about 18% phosphatidylcholine (PC), 14% phosphatidylethanolamine (PE), 9% phosphatidylinositol (PI), 5% phosphatidic acid (PA), 2% minor PLs, 11% glycolipids, 5% complex sugars, and 37% neutral oil (2). Deoiling crude lecithin is considered necessary in making high-purity lecithin products. Acetone is currently used in industry for separating neutral oil and PLs since neutral oil is soluble in acetone whereas PLs are insoluble. Deoiled lecithin contains about 23% PC, 20% PE, 14% PI, 8% PA, 8% minor PLs, 15% glycolipids, 8% complex sugars and still 3% neutral lipids (3). There are about 5% increases in PC, PE, and PI proportions compared with the crude lecithin. Because of the different functional properties of the various PLs classes (4), it is desirable to further fractionate the deoiled lecithin with ethanol or ethanol/water systems. Because PC is relatively more soluble in ethanol than is PI, ethanol extraction yields a PC-enriched fraction

(4). This PC-enriched fraction may be a better o/w emulsifier, whereas PI-enriched fraction could be used as a w/o emulsifier and is used in the confectionery industry (5).

Lecithin recovered from soybean oil that is extracted by extruding-expelling (E-E) process is not solvent treated. There is growing interest in obtaining purified or fractionated lecithin without solvents or chemicals. If ethanol fractionation of lecithin could be conducted without acetone deoiling of the crude gum, the lecithin products obtained might have increased value. Our research hypothesis is that the oil contained in crude gum may act as a co-solvent in ethanol fractionation, and the separation of PC from PI may be improved compared with fractionating deoiled gum.

The PL molecule contains both a lipophilic fatty acyl group and a hydrophilic head group, and this amphiphilic structure makes it a good surface-tension-reducing agent and thus a good emulsifier (6). Critical micelle concentration (CMC) is usually used as an indicator of the effectiveness of surface-active agents (surfactant or emulsifier) (7). Emulsion stability refers to the ability of an emulsion to resist changes in its physical properties over time: the more stable the emulsion, the more slowly the phases separate (8). PC-enriched and PI-enriched fractions have been used as emulsifiers for creating both o/w and w/o emulsions for the emulsion stability test. There is concern about the oxidative stability of PLs, which are believed to be more unsaturated than the soybean oil from which they are recovered from (9). The fractionated products should have different fatty acid composition; the PC-enriched fraction will be more unsaturated and PI-enriched fraction more saturated. It is important to quantify the oxidative stabilities of these products.

In this study, crude lecithin was fractionated and the PC-enriched and PI-enriched fractions were tested for functionality, e.g. surface tension reduction, emulsion stability, and oxidative stability.

## Materials and Methods

*Gum fractionation.* Extruded-expelled oil from commercial soybeans was filtered to remove meal fines. Water (0.3% oil weight) level was metered in the oil stream at about 60°C. The mixture was passed through an in-line static mixer and the gums were allowed to settle in a vessel. Two days later, the oil was pumped out and the crude gum collected. The crude gum was centrifuged at 950 x g for 15 min, about 40% of the weight was removed as oil. The residual gum after centrifugation contained 4% moisture. A factorial experimental design was used, with ethanol concentration (3 concentrations) and solvent:gum ratio (5 ratios) as two factors. About 3 g of gum was weighed into a centrifuge tube; then, ethanol (three concentrations of ethanol: 90, 95, and 100%, and five ratios of ethanol to gum: 0.5, 1.0, 1.5, 2.0, 2.5) was added. The tube was heated in a water bath at 60-70°C for 1 h, stirred every 10 min during heating for maximal dissolution of PC in ethanol, and then the solution was centrifuged at 950 x g for 5 min. The upper clear phase (ethanol phase) was poured into another tube. The residual lower phase was deoiled with acetone according to AOCS Official Method Ja 4-46, procedures 1-5 (10). Both the upper (PC-enriched fraction) and lower phases (PI-enriched fraction) were dried for PLs quantification by HPLC. Treatments were duplicate.

*Fractionation of deoiled and oil-containing gum.* Two types of materials were used to examine the effect of oil on the effectiveness of fractionation: deoiled gum and crude gum.

Crude gum was obtained from the same source and procedures as described above. Deoiling was performed according to AOCS Official Method Ja 4-46, procedures 1-5 (10). Aqueous ethanol (90%) with the ratio of ethanol to gum at 3:1 was used to fractionate PC and PI. The fractionation was conducted using a similar procedure as discussed above. Treatments were duplicated. The fractionation of deoiled gum was designated as sequence 1 (Seq. 1), and the direct fractionation of oil-containing gum was designated as sequence 2 (Seq. 2).

*HPLC quantification.* Beckman Coulter (Fullerton, CA) HPLC system with auto sampler 508, solvent delivery module 126, silica column (250 mm length, 2.1 mm id, from Alltech, Deerfield, IL), and evaporative light scattering detector (ELSD 2000, Alltech) was used for the PLs class composition analysis. Two mobile phases with a gradient program were used: "A" is chloroform/methanol/ ammonium hydroxide (80:19.5:0.5, vol/vol), "B" is chloroform/methanol/water/ammonium hydroxide (60:34:5.5:0.5, vol/vol) (11). Flow rate was 0.3 mL/min. Nitrogen (1.6 L/min flow rate) was used to evaporate the solvent in the heated chamber (50°C).

*Surface tension reduction.* PC-enriched and PI-enriched fractions from ethanol fractionation were deoiled again with acetone to obtain relatively oil-free PLs samples for functionality testing. The concentration of PL aqueous dispersion was calculated based on the total PLs in the purified sample. The PLs were individually dispersed in water at high concentrations and then diluted with water to lower the concentrations. Surface tensions at each concentration were determined with a FACE Automatic Surface Tensiometer (model of CBVP-Z, Tantec Inc., Schaumburg, IL). Surface tension was plotted against concentration. The initial reduction portion and the stabilized or horizontal portion of the curve were analyzed separately to obtain the linear trend of each portion, and the intercept where the

surface tension started to become constant with increase in concentration was considered as CMC. Data were statistically analyzed with the General Linear Model of the SAS program to examine the effects of sample type on CMC (12). Least significant differences (LSD) at $P$ = 0.05 were calculated to compare treatment means. A commercial lecithin (Fisher Scientific, Pittsburgh, PA) with an acetone-insoluble value of 97 was also employed in the surface tension reduction test for comparison.

*Emulsion stability.* Both PC-enriched and PI-enriched fractions as well as commercial lecithin were used as emulsifiers for making w/o and o/w emulsions. For both types of emulsions, two levels of emulsifier relative to the discontinuous phase were used: 5 and 10%. For o/w emulsion, the proportion of oil to water was 1:9; for w/o emulsion, the proportion of water to oil was 2:8. Emulsions were created by dispersing the emulsifier thoroughly in oil; then, water was added and blended with a 50-ml Waring® blender at high speed for 3 min. Emulsions were then transferred to 10-ml pipettes with the bottom sealed. The pipettes were held vertically and the volume of separated discontinuous phase was recorded periodically. The Michaelis-Menton equation was used to model the data and to study the stability profile (13). The reciprocal of percentage of separated oil to total oil was plotted against the reciprocal of time. The maximal percentage of separated oil to total oil (referred to as $P_{max}$) and the time used for reaching half of $P_{max}$ (referred to as $T_{1/2}$) were calculated with the equation. A factorial experimental design was used to examine the effects of emulsifier type and percentage of lecithin on the stability of emulsion with the SAS program (12).

*Oxidative stability.* PL samples were dissolved in mineral oil that should is oxidatively stable. The lecithin was completely dispersed making oxidation more uniform than if lecithin was in a solid state. The mixture was placed in an oven at 90°C for several

days with periodic sampling. A Stamm test (14) was used for quantifying peroxides for both the PC-enriched and PI-enriched fractions. Compared with the Iodometric method, the Stamm test uses much less of the lipid sample. The commercial lecithin was also used for the oxidation test with both the AOCS official Iodometric method Cd 8-53 (10) and the Stamm test so that the reliability of the Stamm test can be validated.

## Results and Discussion

*Effect of ethanol strength and solvent-gum ratio on PLs fractionation.* The yield of the PC-enriched fraction, which is a high-value product used in the pharmaceutical industry, was significantly affected by both ethanol concentration and solvent-gum ratio. There was no significant interaction between the two factors ($P>0.05$). Table 1 shows the $P$ and $LSD_{0.05}$ values from the SAS analysis of PLs content and proportions in PC-enriched and PI-enriched fractions obtained from different ethanol concentrations and ratios. The effects of concentration and ratio on PL fractionation were different. Yields of PC-enriched fractions at those three ethanol concentrations ranged from 20 to 25% (Fig. 1). But at different ratios, they ranged from less than 10% to more than 30%. Table 1 also shows that the PL content in the PC-enriched fraction was not significantly affected by ethanol concentration or by ratio. For the individual PL contents, the ethanol concentration significantly affected the proportion of all three PLs in the PI-enriched fraction as well as PE and PI in the PC-enriched fraction with the highest ethanol concentration resulting in the most PC and the least PI in the PC fraction and the most PI and the least PC in the PI fraction (Fig. 2). Though the ratio only affected PE content in the PC-enriched and PC and PI in the PI-enriched fraction, the higher the ratio of ethanol to gum, the more PI and less PC in the PI-enriched fraction.

Although ethanol concentration gave a statistically significant difference in total yield of PC-enriched fraction, the difference was not great. Ethanol concentration significantly influenced PC and PI fractionation with the highest concentration giving the best fractionation. In this case, the highest PC proportion in the PC fraction and the highest PI proportion in the PI fraction were achieved. Though the ethanol-to-gum ratio significantly affected the production of the PC-enriched fraction, it did not affect the relative contents of PC and PI in the PC-enriched fraction. In addition, the total PL contents in the two fractions did not change significantly with ethanol-gum ratio. Therefore, the highest concentration of ethanol and highest ethanol-to-gum ratio gave the best fractionation and the highest production of PC-enriched fraction.

*Gum fractionation on large scale and effect of oil on fractionation.* Fractionations of deoiled and oil-containing gums are shown in Fig. 3. Seq. 1 represents the process of acetone deoiling the crude gum (245 g) first, removing 160 g of oil, and then ethanol fractionating the deoiled gum resulting in 23 g of PC-enriched and 65 g of PI-enriched fractions. The total PLs in the final products (PC-enriched and PI-enriched fractions) were 36.5 g, accounting for 15% of the original gum. Seq. 2 represents the process of direct ethanol fractionation of oil-containing gum and then acetone-deoiling of the PI-enriched fraction because that oil was mainly remained in this fraction rather than in the PC-enriched fraction. Using this process, 33 g of PC-enriched and 63 g of PI-enriched fractions were obtained while 149 g of neutral oil were removed. Total PLs in the final PC- and PI-enriched products were 47.3 g, accounting for 19.3% of the original gum. The yield of the PC-enriched fraction as well as the total PLs remaining in the final products (i.e., purity) produced from the deoiled gum was less than those produced from oil-containing gum. More PL was lost in Seq. 1 than in Seq. 2,

the reason may be that although PLs are not soluble in acetone, some PLs can still be lost or trapped in the oil when the oil was first removed by acetone. This was confirmed by the difference in the amount of oil washed out by acetone between the two methods. This difference equaled the amount of the PL lost in the final products of Seq. 1. Of the PLs lost during acetone deoiling in Seq. 1, 3.9 g were PC, 4.5 g were PE, and 2.4 g were PI.

Based on the same amount of original gum, oil-containing gum resulted in 10 g more PC-enriched fraction than deoiled gum did with 10% more total PLs in the former than in the latter. The extra PL in the PC-enriched fraction from oil-containing gum was 4.5 g PC, 2.1 g PE, and 0.7 g PI. The two procedures resulted in a similar amount of the PI-enriched fraction, but the total PLs contents were different. The PI fraction from the deoiled gum had 42% total PLs while the fraction from the oil-containing gum had 49% total PLs. The PI-enriched fraction from oil-containing gum had 0.6 g less PC, 2.4 g more PE, and 1.7 g more PI than that from deoiled gum. Fig. 4 shows the percentages of PLs classes in the PC and PI fractions produced from Seq. 1 and Seq. 2. Seq. 2 produced about 5% more PC in the PC-enriched fraction and about 3% more PI in the PI-enriched fraction.

Thus, Seq. 2 of fractionating oil-containing gum is more effective in separating PC and PI, producing more PC in its PC-enriched fraction and more PI in its PI-enriched fraction. The oil contained in the crude gum acted as a co-solvent to trap the PI, improving the separation of PC and PI during ethanol fractionation. In addition, because the PC-enriched fraction obtained from oil-containing gum was not subjected to any undesirable organic solvent processing, it could maintain its "natural" characteristics. Thus, the small soybean processing plants that employ E-E technology not only could avoid the use of

flammable acetone, but also could improve both the quantity and quality of their ethanol-fractionated PC products.

*Functionality of fractionated lecithin products.* Both the PC-enriched and PI-enriched fractions contained only about 40 to 50% of PLs. In order to obtain more purified lecithin products for functionality tests, acetone washing was performed two more times for both PC-enriched and PI-enriched fractions. After this treatment, there were about 92% of PLs in the PC-enriched fraction, but still only about 65% of PLs in the PI-enriched fraction. With TLC analysis, we identified the impurities in these two fractions as lyso-PC, cerebrosides, monoacylglycerol, diacylglycerol, and triacylglycerol. There were still compounds at the sample origin that might have been glycolipids, but we did not confirm this. Except for lyso-PC, all the other components are less polar than PLs allowing them to be more easily trapped in the PI-enriched fraction, which is less polar than the PC-enriched fraction. This result was in agreement with our observation that the spot of lyso-PC of the PC-enriched fraction was relatively more intense than that of PI-enriched fraction, whereas the other impurity spots of the PI-enriched fraction were relatively brighter than those of the PC-enriched fraction. This may result in lower PL content in the PI-enriched fraction because PI had the majority of the impurities. But we cannot explain why these non-polar impurities could not be removed by additional acetone washing. The purities of the lecithin products were considered when conducting functionality tests.

*Surface tension reduction test of the PC and PI fractions.* The surface tension reduction test significantly differed between the two lecithin products. PC-enriched and PI-enriched fractions reduced surface tension drastically at low concentrations and then the surface tension stayed constant with increasing PL concentration (Fig. 5). Linear regression

was applied separately for the initial reduction and the stabilized portions; the intercept point of these two lines is the CMC. Mean CMCs for duplicate surface tension tests for PC- and PI- enriched fractions were 2.67 mg/ml and 0.72 mg/ml, respectively. Statistical analysis showed that there was a significant difference between the CMC of the two fractions (*P*-value was 0.0018) with the least significance difference value being 0.36 mg/ml. Above the CMC, the thermodynamic activity of emulsifier does not increase with the addition of more emulsifier (7). The smaller the CMC of the emulsifier, the better its emulsification ability. The commercial lecithin was employed in the surface tension reduction test for comparing the PC- and PI-enriched fractions. Its CMC was 13.60 mg/ml, which was much higher than the two PL fractions. From our HPLC analysis, this lecithin contained 52% PC, 27% PE, and 21% PI. Its percentage of PC was between those of PC- and PI- enriched fractions, and so was the PI percentage.

The surface tension of the system was reduced to a constant 38 mN/m when CMC was reached by the PC-enriched fraction, whereas it was reduced to 21 mN/m by the PI-enriched fraction. The PI-enriched lecithin had significantly better surface tension reduction capability than PC-enriched lecithin. The lower the surface tension, the larger the amount of new interfacial area that was created for a given amount of energy input (7) and the better emulsification capability of the emulsifier. Considering this degree of surface tension reduction and the CMC value, the PI-enriched fraction should be regarded as a better emulsifier than the PC-enriched fraction. Because the two fractions are all mixtures of PL as well as some other minor components, it is possible that the PLs class proportion as well as the minor components of the PI-enriched fraction contributed to this functional property.

Fujita and Suzuki (15) reported that soy lysophospholipids had different surface tension reduction capabilities when mixed with different fatty acids. Saturated fatty acids, such as palmitate and stearate, reduced surface tension the most. Our previous study illustrated that in soy lecithin, PI was more saturated than PC and PE did. Although the two systems were different -- the former was PL mixed with fatty acids, the latter was different fatty acids esterified in PL -- the contribution of the fatty acids may be similar. The increased palmitate and stearate in the PI-enriched fraction may contribute to the fact that the PI-enriched fraction has better surface tension reduction capability.

*Emulsion stability.* Both PI-enriched and PC-enriched fractions created o/w emulsions. Fig. 6 shows how the percentage of separated oil relative total oil volume changed with time. The shape of the curves resembles the enzyme kinetics curve, which could be expressed by the Michaelis-Menton equation. We used this mathematical model to fit the data and obtained the characteristic parameters, $P_{max}$ and $T_{1/2}$, which were used to compare treatment effects. $P_{max}$ was the index of the maximal breaking of the emulsion and $T_{1/2}$ was the index of the speed of emulsion breaking. SAS analysis showed that $P_{max}$ and $T_{1/2}$ were significantly affected by the type and percentage of lecithin; there was significant interaction between these two parameters. The emulsion created by the PI-enriched fraction broke more slowly and to a less degree than that created by the PC-enriched fraction (Table 2, Fig. 6). These results indicated that the PI-enriched phase performed better than the PC-enriched fraction in o/w emulsion and are in agreement with the results from the surface tension reduction measurement. But our observation is contrary to the results of others, which suggests that the PC-enriched fraction is an excellent o/w emulsifier (5). Commercial lecithin

mentioned above was also used for the emulsion stability test. Fig. 6 shows that it performed similarly to the PC fraction at both the 5 and 10% levels.

For w/o emulsion, the PI-enriched fraction created emulsion at both the 5 and 10% levels of lecithin to water. These emulsions were very viscous, the texture and appearance being similar to yogurt. Because of the thin inner diameter of the pipettes as well as the viscous nature of the emulsion, the separated water was difficult to accumulate; thus, there was no obvious frontier of the separated water phase and it was impossible to record the separated water volume in as short a period as for o/w emulsions rather than estimating it. At 2.5, 20 and 44 h, 12, 60, and 68% of emulsion separated out as oil for the emulsion created with 10% lecithin to oil, while for 5% lecithin to oil, they were approximately 11, 60, and 71%, respectively. The two percentages of lecithin to oil did not result in significant difference in the stability of emulsion. The PC-enriched phase did not create an emulsion at either 5 or 10%. Instead, the lecithin hydrated and settled from the oil, a phenomenon very similar to the degumming of crude soybean oil. These results show that the PI-enriched fraction is better than the PC-enriched fraction when creating w/o emulsion. This corresponds to the results of others. Theoretically, PI has a lower HLB (hydrophilic-lipophilic balance) number, which suggests a good w/o emulsifier (16). So the relatively higher PI content in the PI-enriched phase favors the w/o emulsion formation. The commercial lecithin created viscous and yogurt-like emulsions at 5 and 10% levels, and the separation rates were similar to those of the PI-enriched fraction.

*Oxidative stability.* The peroxide values of the PC-enriched and PI-enriched lecithin determined by the Stamm method fluctuated over the 170-h period at 90°C and did not show a definite trend. The mean and standard deviation of the PC- and PI-enriched fractions are

3.30 ± 2.19 and 2.82 ± 1.68 meq/kg, respectively. It suggests that the lecithin products were not oxidized under our test conditions.

PL is an antioxidants and a synergists (17) or only an antioxidant synergists (18). Because there was no tocopherol detected in our samples, it is likely that the PL behaved as an antioxidants. The antioxidant characteristic of lecithin is believed to relate to metal scavenging ability of the PL (8). The metal ions may be chelated by the anion head groups of PL to prevent their catalyzing oxidation of fatty acid chains. Our results suggest that the antioxidant property of PC- and PI-enriched fractions is similar, and both are good antioxidants. This information is useful because when PC and PI fractions are used for other functionalities, their application could also achieve oxidative stabilization of both themselves and lipids in the system.

To validate the Stamm method, commercial lecithin was oxidized under the same conditions as was done with the fractionated lecithin and peroxide value was measured by both Stamm and standard Iodometric methods. The two sets of PV were similar. This validated the reliability of Stamm test. The standard Iodometric method needs a 5-g sample, whereas a sample of less than 100 mg is required by the Stamm test. Therefore, the Stamm method is a good choice for PV determination when only a small quantity of sample is available. PV decreased over time at 90°C, from 3.21 to 0.58 with the Stamm method and from 2.28 to 0.87 with the Iodometric method (Fig. 7). The same oxidation conditions were used on soybean oil for its oxidative stability test with the Iodometric method, which resulted in increased PV with 34.49 at 48 h, 60.47 at 96 h, and 93.45 at 144 h. The mineral oil in the system did not have an effect on the oxidative stability of soybean oil and lecithins.

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**TABLE 1.**
**$LSD_{0.05}$ and *P* Values for the Effect of Ethanol Concentration and Solvent-Gum Ratio on Fractionation Yield and PL Class Proportion (number of replication = 2)**

| | Yield of PC-enriched fraction | | % of PLs in PC-enriched fraction | |
|---|---|---|---|---|
| | $LSD_{0.05}$ | *P* value | $LSD_{0.05}$ | *P* value |
| Concentration* | 3.56 | 0.0267 | 4.24 | 0.6315 |
| Ratio** | 4.59 | <0.0001 | 5.47 | 0.2949 |

PC-enriched fraction

| | % PC | | % PI | | % PE | |
|---|---|---|---|---|---|---|
| | $LSD_{0.05}$ | *P* value | $LSD_{0.05}$ | *P* value | $LSD_{0.05}$ | *P* value |
| Concentration | 4.41 | 0.2046 | 3.77 | 0.0218 | 1.33 | 0.0256 |
| Ratio | 5.7 | 0.7804 | 4.87 | 0.6307 | 1.72 | 0.0039 |

PI-enriched fraction

| | % PC | | % PI | | % PE | |
|---|---|---|---|---|---|---|
| | $LSD_{0.05}$ | *P* value | $LSD_{0.05}$ | *P* value | $LSD_{0.05}$ | *P* value |
| Concentration | 0.98 | <0.0001 | 2.3 | 0.0128 | 2.29 | 0.0237 |
| Ratio | 1.27 | <0.0001 | 2.97 | <0.0001 | 2.96 | 0.83 |

* Ethanol concentrations: 90%, 95%, and 100%.
** Ratio of ethanol to gum: 0.5,1,1.5, 2, and 2.5.

**TABLE 2.**
**Mean Value and Standard Deviation of $P_{max}$ and $T_{1/2}$ for Emulsion Stability Test for PC-Enriched and PI-Enriched Fractions**

| | 5%*** | 10% |
|---|---|---|
| $P_{max}$ * | | |
| PC-enriched fraction | 0.3260 ± 0.0042 | 0.2655 ± 0.0233 |
| PI-enriched fraction | 0.1705 ± 0.0035 | 0.0360 ± 0.0014 |
| Commercial lecithin | 0.2674 ± 0.0079 | 0.2892 ± 0.0140 |
| $T_{½}$ ** | | |
| PC-enriched fraction | 35.47± 0.93 | 53.59 ± 4.92 |
| PI-enriched fraction | 89.08 ± 15.04 | 551.25 ± 15.91 |
| Commercial lecithin | 12.73 ± 3.92 | 104.52 ± 2.74 |

* $P_{max}$ is the maximal percentage of separated oil volume to total oil volume.
** $T_{½}$ is the time needed for reaching half of $P_{max}$.
*** Percentage of weight of lecithin to weight of oil.

**Figure captions:**

**Fig. 1.** Percentages of ethanol extractable in total gum after fractionated with ethanol at various ethanol concentrations and ratios of ethanol to gum.

**Fig. 2.** Relative percentages of PLs class in PC-enriched and PI-enriched fractions after fractionated with ethanol at various ethanol concentrations and ratios of ethanol to gum.

**Fig. 3.** Diagram of fractionation of deoiled and crude gum as Seq. 1 and Seq. 2.
* Referred to as PC-enriched fraction. ** Referred to as PI-enriched fraction.

**Fig. 4.** PL class percentages in PC-enriched and PI-enriched fractions produced from Seq. 1 and Seq. 2. Seq. 1 was acetone deoiling before ethanol fractionation; Seq. 2 was acetone deoiling after ethanol fractionation. Y-axis designates the percentages of PL class in the total amount of either PC-enriched or PI-enriched fraction.

**Fig. 5.** Surface tension reduction of an aqueous system by PC-enriched and PI-enriched lecithin fractions.

**Fig. 6.** Emulsion stability test with 1:9 of oil to water. A. 5% lecithin in oil, B. 10% lecithin in oil. * Percentage of separated oil volume to total oil volume.

**Fig. 7.** PV test results for commercial lecithin from Fisher Scientific (Pittsburgh, PA) with both Iodometric titration and Stamm methods.

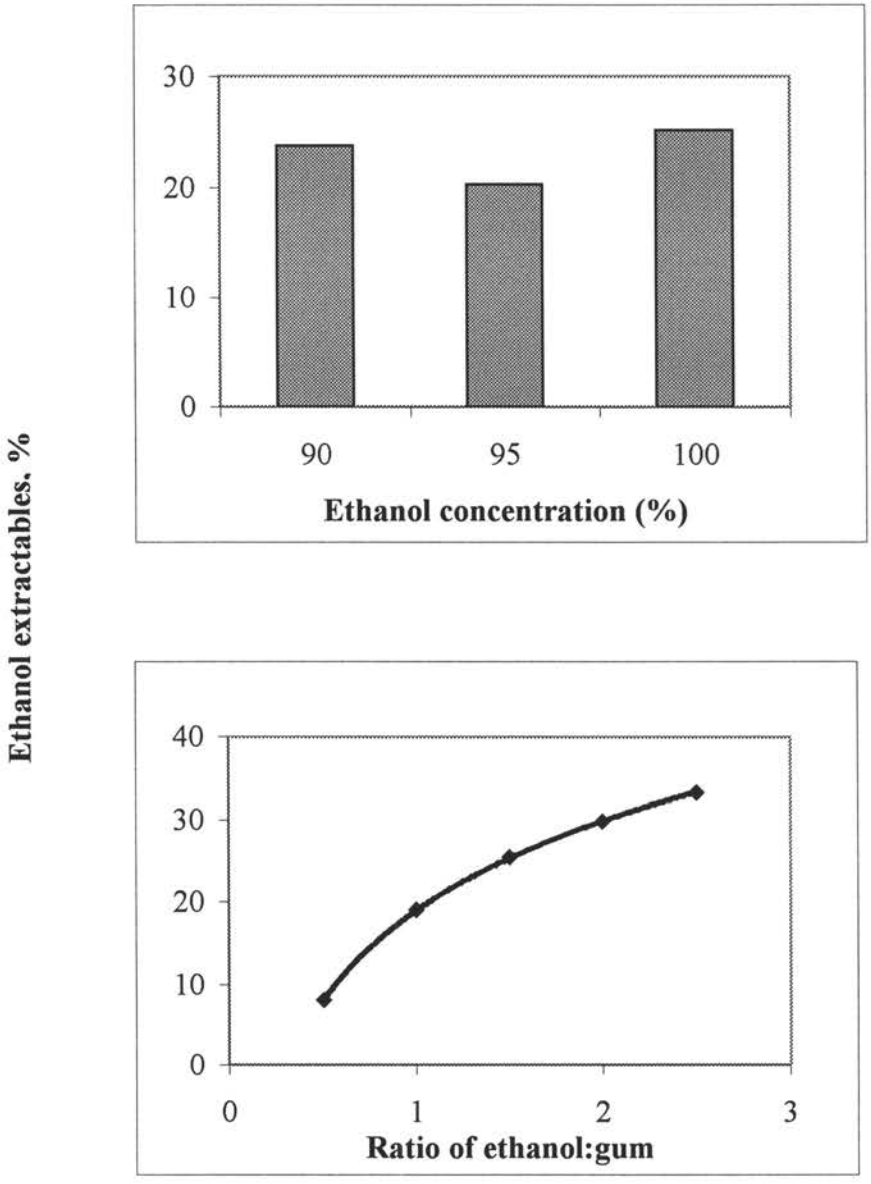
Ethanol extractables, %
30
20
10
0
90
95
100
Ethanol concentration (%)
40
30
20
10
0
0
1
2
3
Ratio of ethanol:gum

**Figure 1**

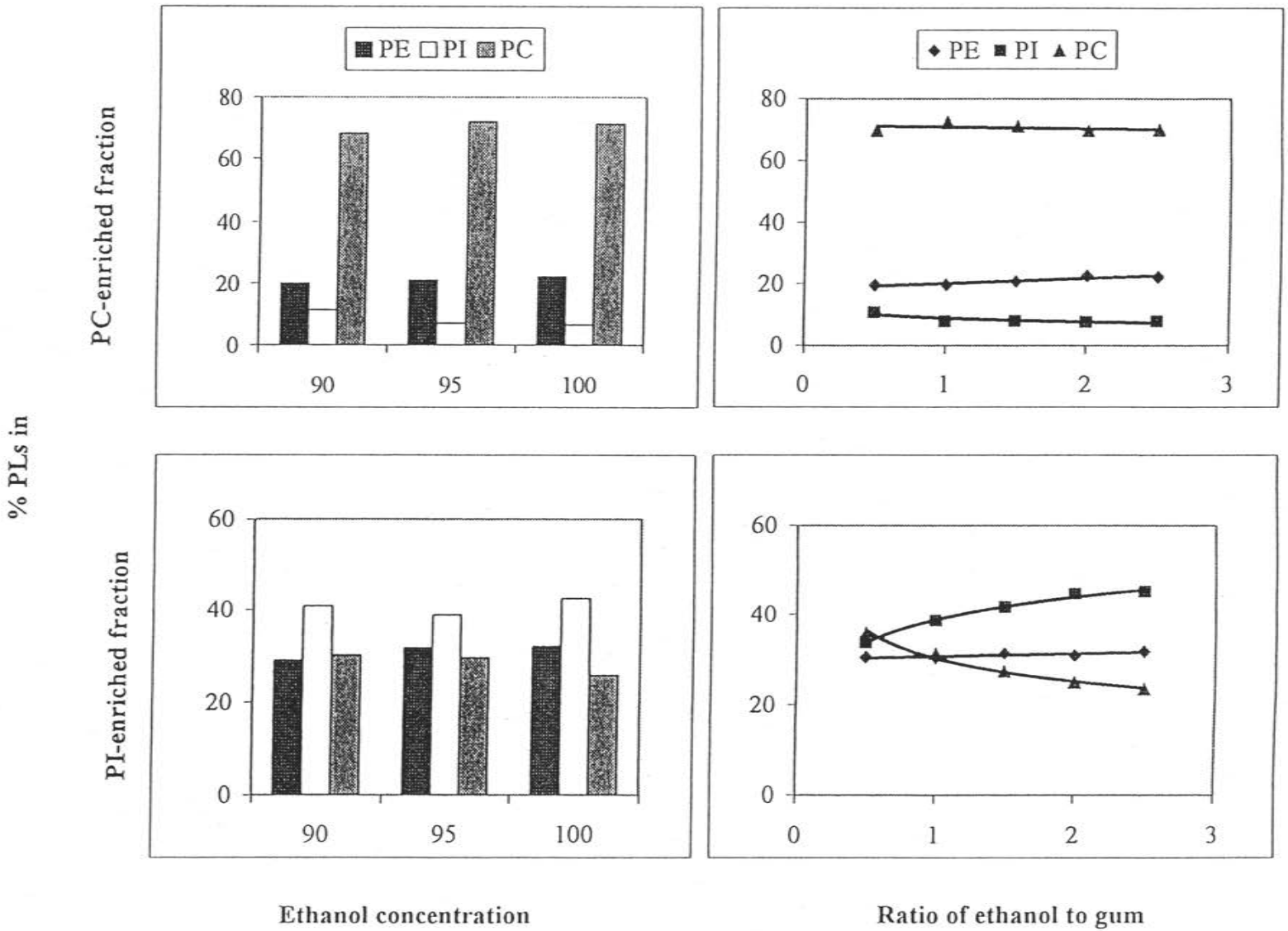
% PLs in
PC-enriched fraction
PE PI PC
80
60
40
20
0
90
95
100
PE PI PC
80
60
40
20
0
0
1
2
3
PI-enriched fraction
60
40
20
0
90
95
100
60
40
20
0
0
1
2
3
Ethanol concentration
Ratio of ethanol to gum

Figure 2

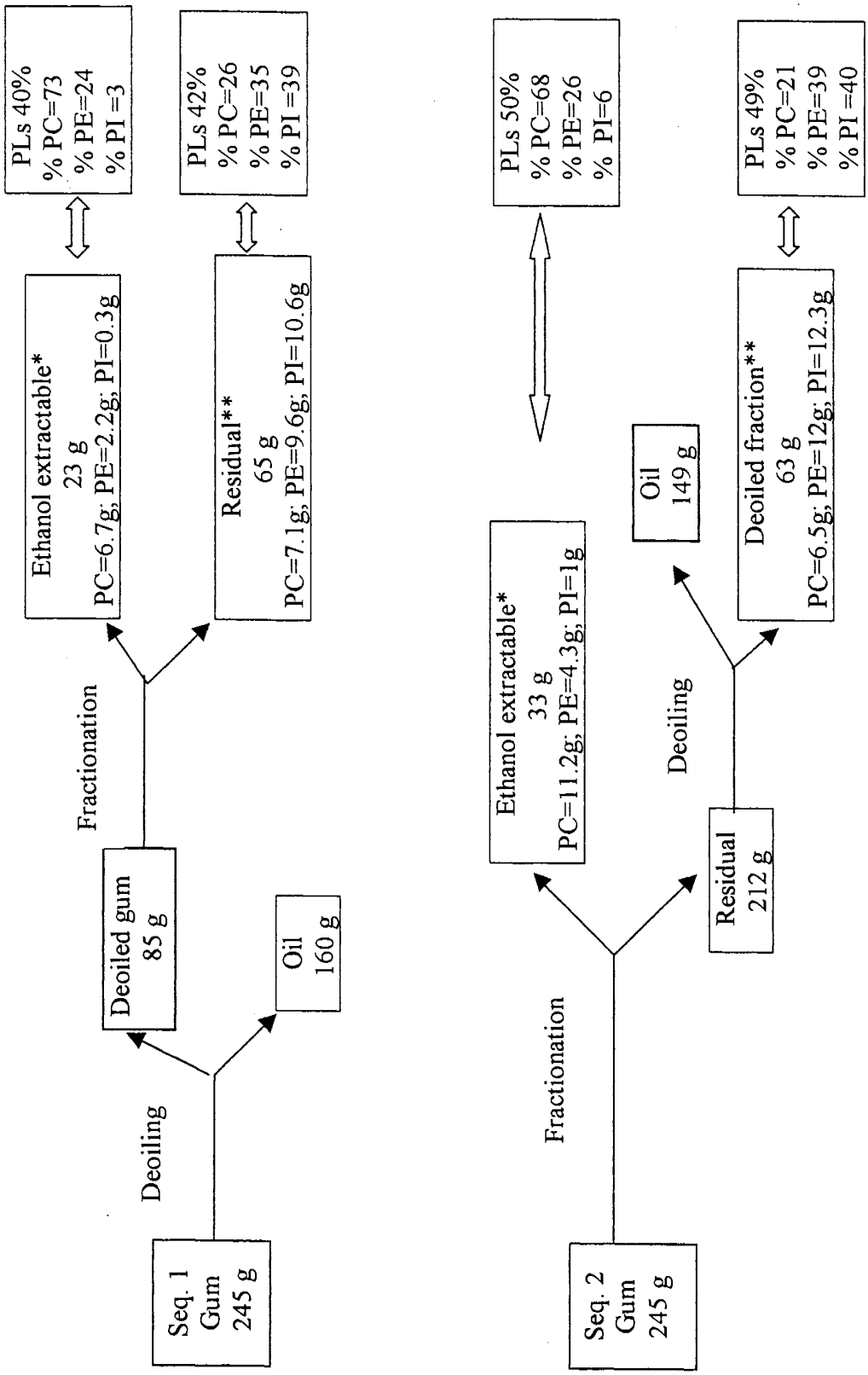
Seq. 1
Gum
245 g
Deoiling
Deoiled gum
85 g
Oil
160 g
Fractionation
Ethanol extractable*
23 g
PC=6.7g; PE=2.2g; PI=0.3g
Residual**
65 g
PC=7.1g; PE=9.6g; PI=10.6g
PLs 40%
% PC=73
% PE=24
% PI =3
PLs 42%
% PC=26
% PE=35
% PI =39
Seq. 2
Gum
245 g
Deoiling
Residual
212 g
Oil
149 g
Fractionation
Ethanol extractable*
33 g
PC=11.2g; PE=4.3g; PI=1g
Deoiled fraction**
63 g
PC=6.5g; PE=12g; PI=12.3g
PLs 50%
% PC=68
% PE=26
% PI=6
PLs 49%
% PC=21
% PE=39
% PI =40

**Figure 3**

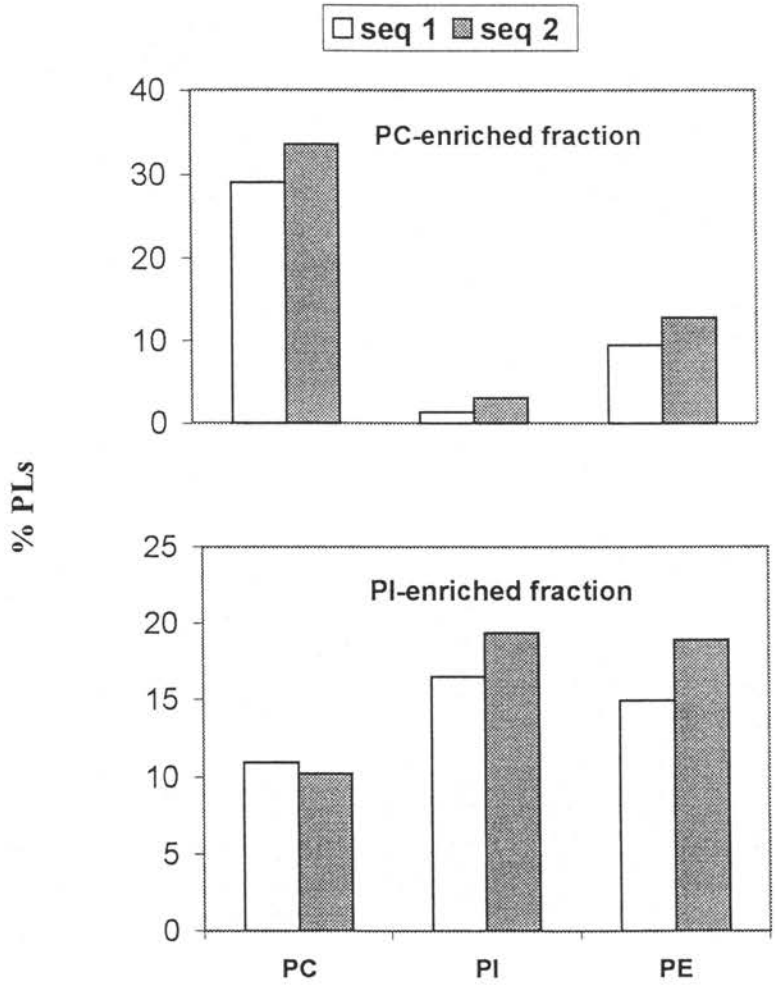
seq 1
seq 2
PC-enriched fraction
40
30
20
10
0
% PLs
PI-enriched fraction
25
20
15
10
5
0
PC
PI
PE

**Figure 4**

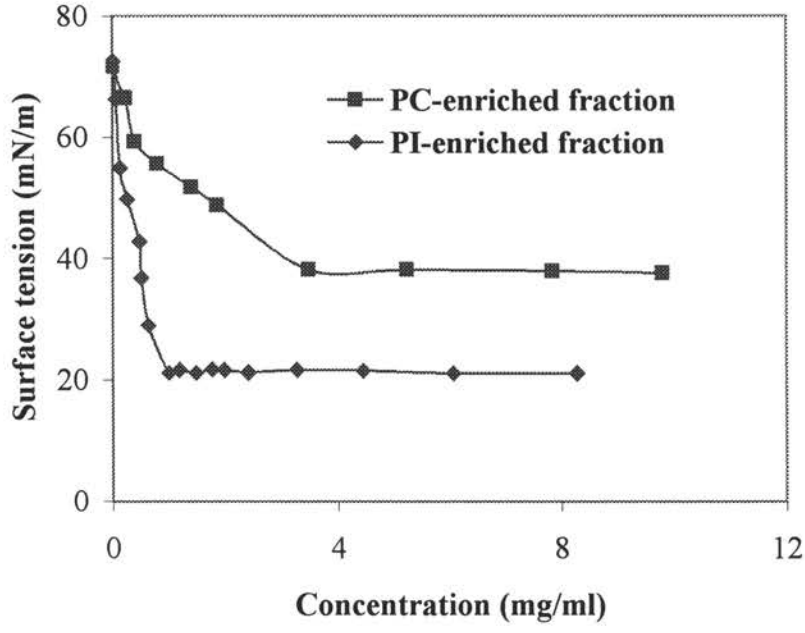
80
60
40
20
0
Surface tension (mN/m)
PC-enriched fraction
PI-enriched fraction
0
4
8
12
Concentration (mg/ml)

**Figure 5**

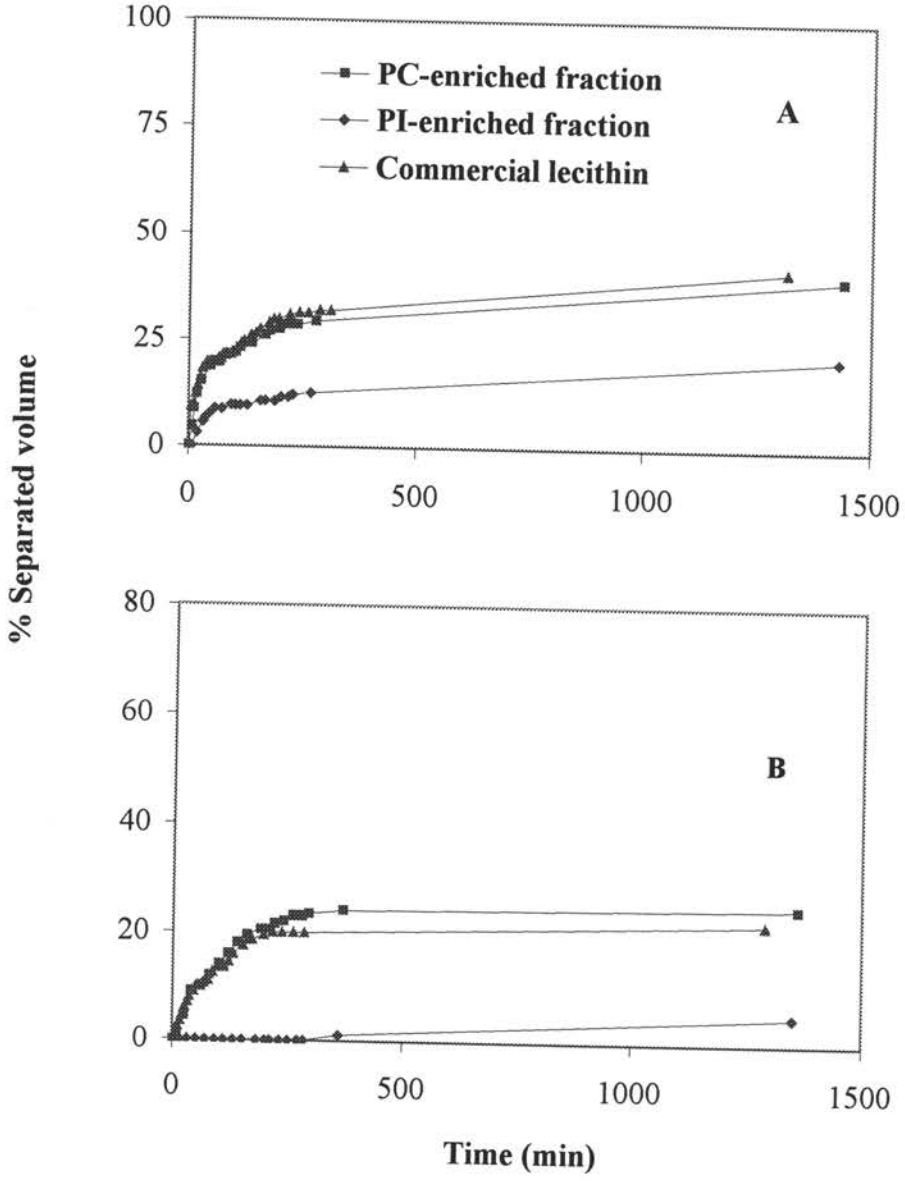
PC-enriched fraction
PI-enriched fraction
Commercial lecithin
A
B
% Separated volume
Time (min)
100
75
50
25
0
80
60
40
20
0
500
1000
1500

**Figure 6**

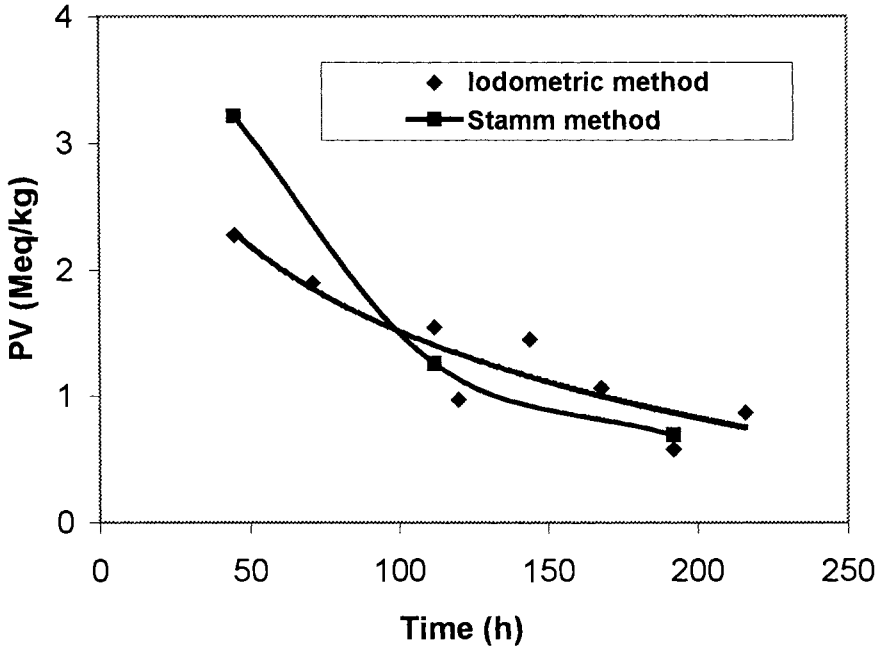
Iodometric method
Stamm method
PV (Meq/kg)
Time (h)
0
1
2
3
4
0
50
100
150
200
250

**Figure 7**

# CHAPTER 4. GENERAL CONCLUSIONS

Soybean lecithin is a product of soybean oil degumming. It is widely used as emulsifier in food, cosmetic, and pharmaceutical industries. The proportion of individual phospholipids (PLs) and their fatty acid (FA) compositions may affect the functional properties of lecithin. In this research, lecithins recovered from four modified soybeans and commodity soybean processed by extrusion-expelling (E-E) and conventional solvent extraction (SE) were analyzed for their PL class proportions and fatty acid compositions. High-performance liquid chromatography (HPLC) with an evaporative light scattering detector analysis demonstrated that the phosphatidylcholine (PC) percentage in E-E lecithin was higher than that in SE lecithin, whereas the phosphatidylethanolamine (PE) content is on the contrary. Gas chromatography (GC) analysis showed that FA compositions of the PLs varied with soybean type. Oil extraction method did not significantly affect FA composition. Critical micelle concentration tested with tensiometer detected differences among the lecithins.

The main PLs in soybean lecithin, PC, PE, and phosphatidylinositol (PI), have different head group, thus different functionality. Fractionation of these PLs is desirable for certain applications. Ethanol was used to fractionate PC and PI, which have different solubilities in ethanol. Various concentrations of ethanol (90, 95, and 100%) and ethanol to lecithin ratios (0.5, 1.0, 1.5, 2.0 and 2.5) were used. Ethanol concentration did not give much difference in total yield of PC-enriched fraction, but it did significantly influence the PC and PI fractionation. Ratio of ethanol-to-lecithin significantly affected the production of PC-enriched fraction, it did not affect the relative contents of PC and PI in the PC-enriched

fraction. Ethanol with ratio of 3 was used for further fractionation analysis. Based on total PLs content, the PC-enriched fraction contained 73% of PC, 24% PE and 3% of PI, whereas PI fraction contained 26% PC, 35% PE and 39% PI. Functional properties of these two fractions were tested for surface tension reduction, emulsion stability, and oxidative stability. PI-enriched fraction had much lower critical micelle concentration than did the PC- enriched fraction, which suggests the PI-enriched fraction has more capability of reducing surface tension. The PI-enriched fraction gave better emulsion stability than PC fraction in both w/o and o/w emulsions. These PLs were very stable to lipid oxidation.

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