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Soybean Phospholipids

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1. Introduction

As soybean phospholipids are coproducts of soybean oil processing, the production of soybean phospholipids rises with the continuous increase of soybean oil yield. Phospholipids have been already applied widely in such fields as medicine, food, agriculture and industry etc., relating to various aspects of everyone's clothing, food, shelter and transportation. New phospholipids products will constantly sprout in large numbers with the development of science and technology.
The authors describe the structure, composition, physical and chemical properties and applications of soybean phospholipids based on the research data in hand. This chapter is focused on the processings of concentrated soybean phospholipids, powdery soybean phospholipids and modified phospholipids as well as the isolation and purification of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA) in soybean phospholipids. The exploration of technologies for isolating and purifying individual molecular species of a certain phospholipids class is now one of the hot and difficult research issues in the world. The breakthrough in these technologies will enormously improve the great development of medicine (e.g. biomembrane bionics, liposomes and intracellular drug carriers etc.) and chemical industry (e.g. aggregation and dispersion of nano materials) etc.

2. The structure, composition and physical and chemical properties of soybean phospholipids

Food Chemicals Codex (FCC) definites phospholipids as follows: Food grade phospholipids are complex mixtures obtained from soybean and other plants consisting of acetone insolubles (AIs) which are mainly phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI).

2.1 Soybean phospholipids structure

Phospholipids mainly include glycerol phosphatides and sphingomyelin. In this chapter, we mainly discuss glycerol phosphatides. The structures of phospholipids are shown in Fig. 1.

2.2 Soybean phospholipids composition

Phospholipids constitute 0.3%-0.6% of soybean seed, or 1.5%-3.0% of crude soybean oil. The phospholipids composition is shown in Table 1. The fatty acid composition of soybean phospholipids is shown in Table 2.

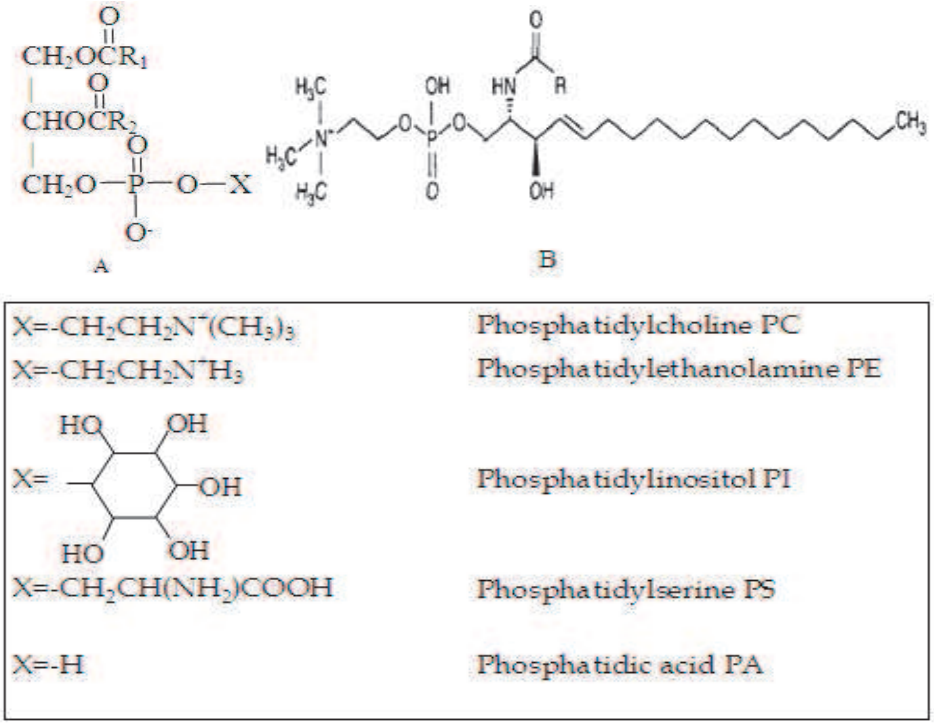

Fig. 1. Structures of phospholipids; A: Glycerol phosphatides structure; B: Sphingomyelin; R1, R2, R: Hydrocarbon chains; Point 'X' is likely composed of structures noted in the box.

Component	Abbreviation	Range(%)		
		Low	Intermediate	High
Phosphatidylcholine	PC	12.0-21.0	29.0-39.0	41.0-46.0
Phosphatidylethanolamine	PE	8.0-9.5	20.0-26.3	31.0-34.0
Phosphatidylinositol	PI	1.7-7.0	13.0-17.5	19.0-21.0
Phosphatidic acid	PA	0.2-1.5	5.0-9.0	14.0
Phosphatidylserine	PS	0.2	5.9-6.3	-
Lysophosphatidylcholine	LPC	1.5	8.5	-
Lysophosphatidylinositol	LPI	0.4-1.8	-	-
Lysophosphatidylserine	LPS	1.0	-	-
Lysophosphatidic acid	LPA	1.0	-	-
Phytoglycolipids		-	14.3-15.4	29.6

Table 1. Composition of Soybean Phospholipids (Szuhaj, 1989)

Fatty acid	Range(%)		
	Low	Intermediate	High
Myristic(C14:0)	0.3-1.9	-	-
Palmitic(C16:0)	11.7-18.9	2.5-26.7	42.7
Palmitoleic(C16:1)	7.0-8.6	-	-
Stearic(C18:0)	3.7-4.3	9.3-11.7	-
Oleic(C18:1)	6.8-9.8	17.0-25.1	39.4
Linoleic(C18:2)	17.1-20.0	37.0-40.0	55.0-60.8
Linolenic(C18:3)	1.6	4.0-6.2	9.2
Arachidic(C20:0)	1.4-2.3	-	-

Table 2. Fatty Acid Composition of Soybean Phospholipids (Szuhaj, 1989)

Soybean phospholipids are by-products of soybean oil refining process. Phospholipids composition can be affected by the oil refining processes and may decrease after frost. The lipase may contribute to phospholipids decrease during storage. Other minor compositions in soybean phospholipids include water, pigment, galactosyl glyceride, glycolipids, carbohydrates, sterols and tocopherol etc.

2.3 Physical and chemical properties

2.3.1 Physical properties

Pure phospholipid is a white solid at room temperature, odorless and colorless. The color of phospholipid may be from light yellow to brown due to refining methods, product categories and storage conditions etc. Non decolored, once decolored and twice decolored are three grades of phospholipid color which is determined by Gardner colorimeter (AOCS official method Td-La-64). The chromaticities are from 9 to 17.

Soybean phospholipids are soluble in aliphatic hydrocarbons, aromatic hydrocarbons and halogenated hydrocarbons solvents, such as ether, benzene, chloroform and petroleum ether etc., and particularly soluble in aliphatic alcohol, for example ethanol. As other non-polar surfactants, soybean phospholipid is insoluble in polar solvent, for example methyl acetate, especially acetone (solubility less than 0.03g/L at 5 degrees Celsius). Phospholipids solubility in methyl acetate and acetone increases when there is a small amount of oil in the phospholipids. PC is soluble in ethanol while PI not. The soluble and insoluble portions of PE in ethanol are about equivalent. The differences of soybean phospholipids solubilities in the above solvents may provide references for isolation, purification and quantification of phospholipids. Soybean phospholipids are soluble in animal fat and vegetable oil, mineral oil and fatty acids, but insoluble in cold animal fat and vegetable oil actually.

The hydrophilic phosphate group and alkaline and hydrophobic hydrocarbon keys in phospholipids molecules help to form a interface between water and oil which lowers the interfacial tension between water and oil and makes them stable colloidal. Soybean phospholipids exist in oil and have obvious hydrophilic colloid property. When mixed with suitable amount of water, phospholipids are isolated from oil. Particularly, in hot alkalescent water (pH>8) the phospholipids are more likely to absorb water and expand and then the colloidal solution is formed. Due to the above property, phospholipids are obtained from crude oil and are widely applied (Lu, 2004).

Phospholipids consist of fluidic and plastic phospholipids. Fluidic phospholipids have the flow property of Newtonian fluid and the fluidity of plastic phospholipids increases with the addition of fatty acids. The viscosity of phospholipids is affected by such factors as AI (acetone insoluble) content, moisture, mineral content, acid value (AV) and various additives for example plant-based surfactant. Generally, high AI or water content results in high viscosity while high AV results in low viscosity. Some bivalent minerals for example Ca^{2+} affect viscosity too.

N-hexane insolubles (HIs) make fluidic phospholipids turbid. The turbidness not only influence the appearance of the products, but also leads to precipitation in long-term storage. The phospholipids also get turbid when the water content is over 1% (Wu, 2001).

2.3.2 Chemical properties

Phospholipids are very unstable when exposed to the air or the sunshine, and color deepening and oxidative rancidity easily happen. However, phospholipids are stable in oil without water. So the oil in concentrated phospholipids can prevent oxidative rancidity and is conductive to the phospholipids storage. Phospholipids are unstable at high temperature. In oil and phospholipids processing, the color of the oil get deeper at 150 degrees Celsius and the odor of phospholipids get worse. Phospholipids decompose at over 150 degrees Celsius.

Hydrolysis of phospholipids occurs upon exposure to strong acid at high temperature. Saponification of phospholipids happens when heated in alkaline ethanol or water solution, and soaps are produced. The salts of phosphoglycerol and inositol phosphate are further heated to be hydrolyzed into glycerol, inositol and phosphoric acid. Free fatty acids and free substances of the above compound are produced after acid hydrolysis or high pressure hydrolysis.

Phospholipids can be hydrolyzed by enzymes. At least four kinds of lipases can cleave ester bonds formed by carboxylic acid and phosphoric acid attached to the glycerol molecule and some of them can only cleave unsaturated fatty acids from phospholipids and can't act on saturated fatty acids. These actions result in production of so called lysophospholipids which have strong effect of hemolysis.

Phospholipids may be modified under certain conditions. The modification reactions include hydroxylation, acetylation, hydrogenation, sulfonation, hydroxyl acetylation and enzymatic modification etc. Modified phospholipids vary in their properties and functions (Lu, 2004).

Phospholipids are regarded as the synergist of antioxidant, and they can synergize or prolong the antioxidation functioin of tocopherol. The synergism of phospholipids differs due to the differences of oil and phospholipids. Mixtures of PE, mixed tocopherol and synthetic antioxidant exhibit the highest antioxidant property (Wu, 2001).

3. Soybean phospholipids processing

3.1 Preparation of concentrated soybean phospholipids

Concentrated soybean phospholipids are products obtained by drying and dehydrating hydrated soybean crude oil foot. Industrial methods preparing concentrated soybean phospholipids include continuous and batch processings.

3.1.1 Continuous processing

The processing steps are as follows: The crude oil is heated to 80 degrees Celsius and then centrifuged and passed through the flowmeter followed by addition of 80 degrees Celsius water of 2% (w/w) of the oil.

Degumming oil and oil foot sediments are produced and they can be separated by centrifugation. The hydrated oil foot should be concentrated immediately to avoid microbial rancidity due to the high moisture and neutral oil content. Oil foot (or mixed with hydrogen peroxide or fluidity agent in advance) is pumped into the agitated film dryer. Phospholipids film is formed under gravity or centrifugal force and the pressure of incoming production materials and flow to the bottom of the vessel while moisture evaporates under high temperature and vacuum conditions. The motionless fluid product is dried at vacuum (726 mm Hg) and 100-110 degrees Celsius for 2 min and then cooled to obtain concentrated soybean phospholipids with less than 1% moisture content. The concentrating procedure should be operated under vacuum as phospholipids are thermosensitive (Ji & Li, 2005).

3.1.2 Batch processing

3.1.2.1 Preheating

Mechanically pressing crude soybean oil is preheated to 80 degrees Celsius after removal of impurities by filtration.

3.1.2.2 Hydration

The amount of water added is determined by phospholipids content in the oil and the changes of phospholipids granules formed during heating and is normally 3.5 times (w/w) the content of phospholipids. The water added is usually boiling or 0.7% hot salt solution is used. The speed of adding water is determined by the water absorption velocity of phospholipids. The faster the latter the faster the former, and vice versa. When adding water, the stirring speed must be fast and is normally 80-100rpm at the beginning and is slowed down 20-30min later when large flocculent phospholipids granules are formed and the stirring is continued for another 20-30min. Then the liquid is left standing still to settle. The supernatant of the upper phase is dehydrated to produce refined oil while the oil foot of the lower phase need to be concentrated to obtain phospholipids products.

3.1.2.3 Concentration

The hydrated phospholipids oil foot is drawn into the concentrating tank by vacuum and subjected to temperature rising and stirring. Vacuum dehydration of phospholipids occurs at about 80 degrees Celsius. When there is slight silk flash while stirring the fluidic phospholipids the moisture content is consistent with the specification. The moisture content is about 5%. Phospholipids after concentration is a brown semisolid and can be used in food, medicine and industry.

3.1.2.4 Decoloration

Decoloration of concentrated phospholipids is needed for preparation of high quality phospholipids. The amount of 30% hydrogen peroxide added to the concentrating tank is 2%-2.5% (w/w) of the concentrated phospholipids. The phospholipids are decolored in the closed tank for 1h at 50 degrees Celsius without vacuum. Then turn on the vacuum pump and heat the mixture to 70 degrees Celsius. Dehydrate until there is no water in the water knock vessel. The decolored phospholipids are light brown.

Mixed fatty acids and mixed fatty acid ethyl ester are added as fluidity agents during the vacuum concentrating procedure to improve the fluidic property of concentrated phospholipids and prevent phospholipids separating with the oil and guarantee the stability of phospholipids products.
The products obtained can flow freely at room temperature. If mixed fatty acids added is inadequate, it will not act as fluidity agent. On the contrary, excess addition of mixed fatty acids may raise the AV of phospholipids and get them rancid. The amount of mixed fatty acids added is usually 2.5%-3% (w/w) of the concentrated phospholipids. The addition of mixed fatty acid ethyl ester does not affect the AV and flavor of phospholipids and can gain high quality products but the cost is high. The amount is 3%-5% (w/w) of the concentrated phospholipids (Ji & Li, 2005).

3.2 Preparation of powdery soybean phospholipids

The applications of concentrated phospholipids are limited due to its high content of neutral oil, fatty acids and other substances and its low purity and off-flavor formation. Refining and purifying processings are needed to consistent the phospholipids products with the high purity and non off-flavor specifications of functional food material.
Methods of producing high purity phospholipids from concentrated phospholipids include solvent extraction, ultrafiltration purification, supercritical carbon dioxide extraction etc. So far, acetone solvent extraction is the most widely used method in industry.

3.2.1 Solvent extraction

3.2.1.1 Preparation of powdery phospholipids of one kind of purity from one kind of materials

The acetone solvent extraction theory is isolating phospholipids from oil by precipitation due to the fact that water and oil is soluble in acetone while phospholipids not.
30% hydrogen peroxide is pumped into the closed agitated container with the amount of 2%-3% (w/w) of the concentrated phospholipids. Concentrated phospholipids are pumped into the above container while stirring with the rotate speed of 30-40rpm. Decoloration occurs after the temperature reaches 60 degrees Celsius with a processing time of 6h. After that, the temperature is raised up to 70-75 degrees Celsius and decolor for 0.5h to decompose residual hydrogen peroxide into water. The decoloring procedure is optional due to the product requirements.
Acetone with purity above 98% is pumped through a flowmeter into the closed agitated container. Concentrated phospholipids with acetone residues of the amount of 1:10 (w/w) are pumped into the above container. Stir for another 20-30min with the speed of 80rpm. After that, the liquid is statically sedimented for 0.5h and the upper acetone extract is discharged. The above procedure is repeated three more times with each time a 5:1 ratio (w/w) of acetone to concentrated phospholipids and prolonging sedimentation time. The total amount of acetone is 25 times (w/w) that of concentrated phospholipids.
Phospholipids settle down at the fourth time is discharged and centrifuged. The diameter of the centrifuge rotor is 800mm and the rotate speed is 1200-1600rpm. Centrifuged phospholipids go directly into the lower closed agitated-container. Acetone of 2 times (w/w) the weight of the concentrated phospholipids is pumped into the same container while stirring (80rpm). The extraction procedure lasts 0.5h and then the liquid is discharged and centrifuged to produce phospholipids with 25%-50% (w/w) acetone. The phospholipids

are fed into the double conic dryer with the amount of 1/3-1/2 of the whole dryer volume. The drying parameters are as follows: drying temperature 50-55 degrees Celsius, rotate speed 10rpm, vacuum -0.083--0.09 MPa, time 4-6h. Then light yellow powdery phospholipids without acetone residue are obtained and weighed for packaging.
The above method can be applied to prepare powdery phospholipids from such various raw materials as soybean, rapeseed, peanut and corn etc. as well as concentrated phospholipids prepared from hydrated oil foot and alkalized oil foot. The powdery phospholipids produced have a phospholipids content of 90%-98% due to the quality of the concentrated phospholipids (Liu & Yang et al., 2006; Liu & Feng et al., 2006; Liu, 2007).

3.2.1.2 Preparation of powdery phospholipids of various purities from one kind of materials

In acetone solvent extraction, the phospholipids purity increases with the increase of acetone amount and extraction times. The increase of phospholipids purity results in longer time needed to settle the whole phospholipids in acetone solution.
If the purity of the powdery phospholipids obtained in 3.2.2.1 is 97%-98%, half of the phospholipids will be settled in 0.5-1h in the fourth extraction while the other half in 4h. The upper phospholipids solution of acetone is discharged when the time has passed 2.5-3h and centrifuged and dried. The purity of the phospholipids produced can reach up to over 99%.
Acetone of 2 times the weight of concentrated phospholipids is added into the extraction tank with agitation. The extraction time is 0.5h and static settle time is 1.5-2h. The upper phospholipids solution of acetone is discharged and centrifuged and dried to obtain phospholipids product with purity of over 95%.
The residual liquid is discharged, centrifuged and dried to produce phospholipids product with purity of about 90% (Liu & Ma, 2011).
This method can produce phospholipids products with various purities due to the product purity obtained in 3.2.2.1 and discharging time to meet the market requirements, and make the best use of the materials.

3.2.2 Supercritical carbon dioxide extraction

Extraction temperature, pressure and time are important technological parameters of supercritical carbon dioxide extraction. Extraction yield increases with the increase of one of the parameters in a certain range while the other two conditions remain unchanged. However, there are also problems of increased cost, power consumption and unsafe factors. Generally, the extracting effect is rather good at 50 degrees Celsius and 20MPa for 5h.
Supercritical carbon dioxide extraction used to extract soybean phospholipids has significance for the industrial application and is an applicative technology with wide prospect as it has the advantages of simple, non solvent residue, safe and reliable and high purity product and it consists with the trend of current green chemical technology (Shi, 2005).

3.2.3 Ultrafiltration purification

The crude phospholipids are subjected to derosination and dissolved in solvents and then passed through ultrafiltration film with certain pore size. Components of suitable sizes pass through the membrane and are isolated.
Ultrafiltration lecithin introduced by ADM（Archer Daniels Midland Co.） which has the property of dry, easy to be mixed with other materials, high quality and high purity is produced by removing the glycerides in phospholipids by ultrafiltration. Ultrafiltration

lecithin can be precisely quantified and conveniently used. In certain situation which has strict requirements for flavor ultrafiltration lecithin is precious as it has good flavor (Shi, 2005).

3.3 Preparation of modified soybean phospholipids

3.3.1 Chemical modification

3.3.1.1 Hydrogenation

After hydrogenation, the unsaturated double bonds of the phospholipids are saturated to improve the stability, oxidative stability, color and odor of the phospholipids. Hydrogenated phospholipids are mainly used in cosmetics, dyes and lubricants.

Powdery soybean phospholipids are dissolved in the mixture of dichloromethane and ethanol (3:1, v/v) with a 1:6 (w/v) ratio in the stainless steel autoclave. A 5% palladium/carbon catalyst is added into the autoclave followed by leakage checking. Then the air in the autoclave is displaced by hydrogen for several times. The reacting parameters include a temperature of 50 degrees Celsius, a pressure of 0.6MPa, a stirring speed of 300r/min, and a reacting time of 3h under constant temperature and pressure. After reaction, the temperature and pressure are reduced. The catalyst is removed and recycled by centrifuging the reaction product. 30% hydrogen peroxide with the amount of 5% (w/w) is added into the liquid portion to decolor and the solvent is removed by rotate evaporation at 55 degrees Celsius. Light yellow solid hydrogenated soybean phospholipids are obtained after vacuum drying at 70 degrees Celsius for 8h. It may be better to use pure ethanol as solvent than the mixture of dichloromethane and ethanol when hydrogenating phospholipid that is soluble in ethanol such as PC (Huang et al., 2003).

3.3.1.2 Acetylation

PE is transformed into N-acylphosphatidylethanolamine after acetylation, and its 'zwitter ion' structure is modified to obtain improvements in Hydrophile-Lipophile Balance (HLB) value, thermostability, oil in water emulsifying ability and viscosity property. Meanwhile, N-acylphosphatidylethanolamine's large solubility in acetone facilitates isolation and purification of phospholipids. Acetylation with acetic anhydride is used to produce acetylated phospholipids in industry.

Considering acetylated phospholipids are mainly applied in food processings, direct heating (noncatalytic) acetylation process is adopted to produce food grade acetylated soybean phospholipids. Acetic anhydride is added into crude phospholipids with the amount of 1%-4% (v/w) due to the PE content in phospholipids and the amino conversion rate. The process requires temperatures of 60-70 degrees Celsius and stirring reacting time of 1h-1.5h. After acetylation, the mixture is neutralized with sodium hydroxide or potassium hydroxide of certain concentration and then dried under vacuum. The specifications of acetylated phospholipids are: free amino 0.7%-1.7%, pH 6.5-8, and HLB value 5-6 (Xu et al., 2008).

3.3.1.3 Hydroxylation

The hydroxylation theory is that two hydroxyls are introduced into the double bonds of the unsaturated fatty acids of phospholipids molecules, i.e., crude phospholipids react with hydrogen peroxide with the existence of organic weak acid such as lactic acid to hydroxylate the unsaturated bonds in phospholipids and oil. The ethanolamine group of PE is also modified. The obvious hydrophilic property makes modified phospholipids more easily disperse in cold water. The degree of hydroxylation modification is controlled by the

amount of hydrogen peroxide added and usually measured by the drop in iodine value (IV). The products with 10%-25% drops in IV have good water dispersibility and oil in water emulsifying property. The emulsifying property decreases and hydrophilic property increases with the increase of drop in IV, but the cost rises too.

Phospholipids hydroxylation processes include such various methods as 'lactic acid + hydrogen peroxide + phospholipids', 'acetic acid + hydrogen peroxide + phospholipids', 'peracetic acid + phospholipids' and 'basic potassium permanganate + phospholipids' etc., which belong to alkyleneortho-dihydroxylation and have various hydroxylation effects. In industrial production, the 'lactic acid + hydrogen peroxide + phospholipids' process is generally adopted as it's a mild method with no problems of the three wastes(waster gas, waster water and industrial residue) and meets the food grade requirements. 75% lactic acid and 30% hydrogen peroxide with the amount of 1%-3% and 5%-15% (v/w), respectively, are added into crude phospholipids. The reaction is carried out at 50-70 degrees Celsius with stirring for 1h-3h. The mixture is neutralized with sodium hydroxide of certain concentration and then dried under reduced pressure until a less than 1% moisture content is reached. The specifications of hydroxylated phospholipids include: drop in IV 10%-25%, pH 6.5-7.5, HLB value 9-10 (Xu et al., 2008).

3.3.1.4 Acetyl-hydroxylation

Acetyl-hydroxylation refers to acetylation of phospholipids followed by hydroxylation, i.e., double modification. Hydroxylation occurs between phospholipids and hydrogen peroxide with the help of acetic acid produced by acetylation. The procedures are as follows: acetic anhydride is added into the crude phospholipids with the amount of 1%-4% (v/w) due to the PE content in phospholipids and the amino conversion rate. The reaction is carried out at 60-70 degrees Celsius for 1h-1.5h with stirring. Then hydrogen peroxide of 5%-15% (v/w) is added. Temperatures of 60-75 degrees Celsius and stirring reacting time of 1h-3h are required. At last the mixture is neutralized with sodium hydroxide of certain concentration and then dried under reduced pressure until reach a less than 1% moisture content. The specifications of acetyl-hydroxylated phospholipids are drop in IV 10%, free amino no more than 1.65%, pH 7-8, HLB value 9-10 (Xu et al., 2008).

3.3.1.5 Hydroxyl-chlorination

100 portions of soybean phospholipids are dissolved in 300 portions of n-hexane. Sodium hypochlorite of 22.5% (w/w) of the total phospholipids is added and the pH is adjusted to 4.5 with acetic acid. The reaction is carried out at 50 degrees Celsius for 1h with stirring. The mixture is washed 3 times with each time 100 portions of water is used. The upper phospholipids solution is evaporated to recycle solvent and obtain hydroxyl-chlorinated soybean phospholipids. The emulsion stability, dispersibility and wettability are improved enormously compared with that of non-modified phospholipids (Xu et al., 2008).

3.3.1.6 Sulfonation

The most likely positions for sulfonation are the double bonds of long chain unsaturated alkanes and α-carbon near ester bonds. When sulfonation of phospholipids including PC, PE, PA and PI etc. happens, the position which is most likely to be introduced with active group is hydroxyl of PI. That is to say, sulfonation occurs on double bonds while esterification (sulfation) occurs on hydroxyls. The double bonds in products will diminish or vanish if sulfonation occurs on double bonds totally or partly. The decrease in unsaturation of sulfonated soybean phospholipids results in the drop of IV. So we can determine whether sulfonation on double bonds happens or not by measuring IV.

There have been a lot of reports on sulfonation and sulfation of phospholipids, but maturer method is sulfur trioxide gas phase continuous film sulfonation which is developed in China. The film sulfonation pipe need heat preservation jacket. The parameters of the sulfonation process are a feed temperature of 40 degrees Celsius, a sulfur trioxide/air flow rate of 1.5m^3/h and a protective wind flow rate of 0.25m^3/h. Continuous sulfonation happens in film sulfonator followed by neutralization with alkali and decoloration with 5%-20% hydrogen peroxide. The sulfonated phospholipids with a 4% sulfur trioxide binding amount and 6-8 pH exhibit such properties as light color, hydrophilic property, emulsifying property and good permeability.

Sulfonation provides soybean phospholipids with special properties and raises the HLB value from 1-2 to 12-16. The physical and chemical properties of phospholipids are improved enormously to facilitate the wide applications of phospholipids as fatliquoring agent, flotation agent and emulsifying agent in leather, pharmacy and farm chemical etc. (Zhang et al., 2004).

3.3.1.7 Alkoxylation

Alkoxylation technology including ethoxylation and propoxylation etc. is a main technology producing nonionic surfactant. It is carried out by addition-condensation reaction of oxirane or epoxypropare with initiators (aliphatic alcohol and nonyl phenol etc.), and the initiator-ethoxylation or initiator-propoxylation products are obtained.

Alkoxylated phospholipids are obtained by addition-condensation reaction of alkoxylated reactant such as oxirane and phospholipids containing hydroxyl such as PE and PI. As hydrophilic oxethyl groups are introduced into the polar end of PE and PI molecules, the HLB value and hydrophilic property are increased and the oil-in-water emulsifying ability improved. As with sulfonation and hydrogenation, the alkoxylation process is very complex and the products are mainly used in non-food industry.

There are not many manufacturers producing this kind of products. R & R551 is the representative ethoxylated soybean phospholipids of ADMC. The ethoxylated phospholipids have a 12.5 HLB value.

According to patents that have been made public and reports related, the soybean phospholipids alkoxylation process is mainly as follows: addition reaction occurs between phospholipids (PE and PI) and alkoxylation reactant (oxirane, epoxypropare and glycidol etc.) and alkoxylated soybean phospholipids are produced. For example, 23.5 pounds of oil-containing soybean phospholipids are dissolved in 15 pounds of dimethylbenzene. 4.5 pounds of oxirane is added. The reaction is carried out at 100 degrees Celsius and 0MPa for 3h followed by removal of solvents. The ethoxylated soybean phospholipids which are resinous, insoluble in dimethylbenzene and soluble in water are obtained (Xu et al., 2008).

3.3.2 Enzymatic modification

Chemical modification of phospholipids improves their emulsifying and hydrophilic properties, but damages natural structure of phospholipids as well. Enzymatic modification exhibits such advantages as no need for purifying the reactant, mild reacting conditions, fast, complete, less by-products, exact action position of enzymes and easy to obtain etc. Phospholipases including phospholipase A_1, A_2, C and D can catalyze various hydrolysis of phospholipids as well as esterification and interesterification reaction with the existence of certain acyl receptor and donor to change or modify the structure of phospholipids which will gain different structures and applications. Phospholipase A_2 and D are used in industries while the other enzymes are on the experimental status (Gu et al., 1999).

3.3.2.1 Phospholipase A_1 and number 1,3-position specific phospholipase

Phospholipase A_2 can specifically hydrolyze the acyl at number Sn-1 position of natural phospholipids. But acyl at number Sn-2 position can be easily transferred onto the thermodynamically stable number Sn-1 position and this results in producing of the same products as with phospholipase A_2. The source of phospholipase A_1 is very limited.
Phospholipase with number 1,3-position specificity can selectively hydrolyze acyl at number Sn-1 position of phospholipids, and can replace phospholipase A_1. Number 1,3-position specific phospholipase can directly catalyze interesterification of phospholipids and fatty acids or oleic acid in organic solvents to produce new phospholipids. For example, Lipozyme IM20 can catalyze the intersterification of PC and fish oil fatty acids with 45% eicosapentaenoic acid (EPA). The parameters are: enzyme amount 1.5% (w/w), the optimal ratio of phospholipids to fatty acids 1:2 and the optimal organic solvent n-hexane. The binding ratio of EPA at number Sn-1 position is 17.7%. Polyunsaturated fatty acids such as EPA and docosahexaenoic acid (DHA) which are good for cardiovascular and cerebrovascular health can be attached to phospholipids and obtain better digestion and absorption properties than that with triglyceride through this kind of reaction (Gu et al., 1999).

3.3.2.2 Phospholipase A_2

Phospholipase A_2 (EC 3.1.1.4) specifically catalyzes the hydrolysis of acyl at number Sn-2 position of phospholipids to produce lysophospholipids and fatty acids. Modified soybean phospholipids exhibit obviously improved hydrophilic and emulsifying properties. They can maintain good emulsifying property under conditions of high or low temperatures or low pH or various salt concentrations. Lysophospholipids are applied in bakery food. They form complexes with amylose and retard aging of breads effectively. Lysophospholipids are two times the price of ordinary phospholipids but they have the advantages of smaller dosage, better effect, oxidative stability and antibiotic property. They are industrially produced in Japan and America. The process is as follows: phospholipids are subjected to moisture content adjustion previously and then added into the solutions with phospholipase A_2 of 0.02% (w/v) and calcium chloride of 0.3% (w/v) with stirring. The temperatures are 50-55 degrees Celsius and the reacting time is 7h-9h. The hydrolyzing degree reaches 35%-40% when the acid value (AV) is in the range of 33-30. The following procedures are required to obtain powdery lysophospholipids: concentration under reduced pressure, pressure filtration, washing with acetone, solvent removal under reduced pressure and vacuum drying (Song et al., 2007).

3.3.2.3 Phospholipase C

Phospholipase C acts on phospholipids to produce diglyceride, phosphoinositide, phosphocholine, phosphoethanolamine and phosphoric acid etc. Diglyceride is a bioactive substance which acts as the second messenger in cell signaling transmission and affects the cell metabolism. There are three kinds of specificities of microbial phospholipase C: the first one specifically hydrolyze PI into diglyceride and cyclic phosphoinositide; the second one specifically hydrolyze sphingomyelin and the third one has relatively wider specificity and takes PC as the optimal substrate (Song et al., 2007).

3.3.2.4 Phospholipase D

Phospholipase D (EC 3.1.4.4) can hydrolyze PC into phosphatidic acid and choline. In microwater system with alcohol, phospholipase D can catalyze transacylation which results

in exchange of primary or secondary hydroxyl of some molecules with ethanolamine or choline groups of phospholipids and formation of new phospholipids. This character is called phospholipids' transfer characteristic or base exchange reaction of phospholipase D (Song et al., 2007).

4. Extraction and isolation of soybean phospholipids

4.1 Soybean phosphatidylcholine (SPC)

4.1.1 Organic solvent extraction

Fractions in soybean phospholipids are isolated due to their solubilities' differences in organic solvents. PC exhibits large solubility in lower alcohol (C1-C4) whereas PE and PI have small solubilities. PC- and PI-enriched products can be obtained by their solubilities' difference. When treated with lower alcohol, PC in deoiled phospholipids is soluble in alcohol leaving insoluble matter consisting mainly of PE and PI. The ratio of PC to PE increases from 1:1 (w/w) in raw material to 3:1 (w/w), and even to 12:1 (61% PC, 5% PE).

Better isolation effect on PC can be obtained by isopropanol. Mixtures of high-purity phospholipids and isopropanol with the ratios of 8:157-16:157 (12:157 is optimal) is added into the agitated- and refluxed-closed container. The extraction is conducted in thermostatic water bath or cryohydrate bath with isopropanol volume fraction of 95%-100% (100% is optimal) at -5-15 degrees Celsius (-5 degrees Celsius is optimal) for 5min-11min (5min is optimal). After the extraction, the mixture is filtered, evaporated to remove isopropanol and dried to obtain product with mass fraction of PC increased from 25.6% in raw material to 66.8%. Isopropanol extraction is the most commonly employed step to obtain high PC-containing phospholipids from deoiled phospholipids (An et al, 2001).

4.1.2 Lower alcohol with salt or acid extraction

It's also an effective method to fractionate phospholipids with the property that phospholipids can react with some salts or acids and precipitate. This method is more promising than organic solvent extraction as metal ions or acids can 'recognize' phospholipids molecules more effectively than solvents.

100g of phospholipids containing 45% PC is dissolved in 1L ethanol of 95% before addition of 4.5g of zinc chloride. The light yellow phospholipid-zinc chloride compound precipitate formed is centrifuged, decomposed with 250ml freezed acetone under nitrogen to obtain 36.7g of phospholipids containing 99.6% PC (Ni, 1995).

4.1.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a rapidly developed new technology in recent years. Supercritical fluids most commonly used are carbon dioxide, ammonia, ethylene, propylene and water etc. Carbon dioxide is most frequently used due to its following properties: critical temperature and pressure easy to get, stable chemical properties, non-toxic, odorless, non-corrosiveness and reusable. Supercritical carbon dioxide extraction (SCE) is a method with bright prospect as it can obtain high purity products with no solvent residues and maintain the nutritional and functional properties of the products and need simple process, single equipment and low cost (Guan et al., 2005).

Tekerikler et al. (2001)obtained phospholipids containing 91% PC after SCE of deoiled phospholipids with 10% ethanol as entrainer at 17.2MPa and 60 degrees Celsius. Increasing the pressure to 20.7MPa increased the extraction yield and PC content (95%). Increasing the

temperature to 80 degrees Celsius decreased the extraction yield and PC content which was attributed to decrease of solubility and selectivity of solvents to PC as the solvents density decrease at high temperatures.

4.2 Soybean phosphatidylinositol (SPI)

PI causes concern as it is involved in the transmission of messages in the cell. PI plays and important role in maintaining normal physiological functions of central nervous system, especially in regulating calcium homeostasis. PI on cell membrane can be hydrolyzed by phospholipase C into 1,4,5-triphosphate inositol that goes into intracellular aqueous phase as the second messenger and 1,2-diacylglycerol that stays in the cell wall. These two substances synergetically induce cell reactions such as contraction, secretion, metabolism and proliferation etc.

Soybean phospholipids are rich in PI. PI is a white amorphous solid with its sodium salt a crystal and is wet-sensitive. PI is soluble in water, chloroform and benzene, slightly soluble in methanol, diethyl ether and petroleum ether, insoluble in acetone, ethanol and water. It can be easily oxidized upon exposure to the air (Deng et al., 2003).

4.2.1 Solvent method

The solvent method is conducted to isolate and purify phospholipids and increase the content of a certain constituent with single or mixture of such solvents as methanol, ethanol, isopropyl ketone, acetone, n-hexane and chloroform etc.

The deoiled soybean phospholipids are extracted with appropriate amount of ethanol. The induced ethanol-insoluble phase is vacuum dried to obtain a mixed phospholipids with more PI and less PC which are dissolved in n-hexane before adding 55% ethanol with sodium acetate. The mixture is put into the separating funnel, shaked, allowed to rest and layered. The same procedure is carried out again except that the 55% ethanol is sodium acetate free. The PI obtained is 40%-50% pure. If sodium acetate is replaced by aqueous ammonia with a 8.0 pH and the ethanol concentration increased to 90%, PI of 85% pure can be obtained with the same method.

Purer PI can be obtained by some chemical reaction methods. Soybean phospholipids containing 40% mixed phospholipids are dissolved in such organic solvents as anhydrous pyridine, acetonitrile, dimethyl formamide (DMF) and dimethylsulphoxide (DMSO) etc. Chloride dimethyl tertiary butyl silicon, chloride trimethyl silicon or allyl bromide are added into the mixture to protect hydroxyls of PI by reacting with them. Then PI is isolated from the mixture with solvents such as acetone or ethanol-acetone and hydrolyzed by alkali or acid at room temperature to remove the blocking groups and recover the hydroxyls of PI. PI obtained this way is 98% pure and applied in treating of central nervous system disorder (Deng et al., 2003).

4.2.2 Column chromatography

The phospholipids mixture is dissolved in the mixture of chloroform and methanol in the 1:1 (v/v) ratio before adding aluminium oxide. The eluate contains PC, lysophospholipids, neutral lipids and glycolipids etc. Residues are washed and extracted with the mixture of chloroform, methanol and 1% hydroxyl ammonium acetate in the 1:1:0.3 (v/v/v) ratio and the eluate is loaded on silica column of which the dimension is 30cm. Neutral lipids are eluted with chloroform; glycolipids and PE are sequentially eluted with chloroform and

methanol in the 80:20 (v/v) ratio; PE is further removed with chloroform and methanol in the 20:80 (v/v) ratio; PI is finally eluted with chloroform, methanol and 25% ammonia in the 80:20:5 (v/v/v) and 65:25:5 (v/v/v) ratios. The PI-containing fraction is evaporated and dried to obtain PI of no less than 98%-99% pure (Deng et al., 2003).
Column chromatography can obtain high purity PI but the long time needed and the use of complex solvent mixture reduce its feasibility in the commercial world.

4.2.3 Enzymatic method

Phospholipids can be hydrolyzed by such phospholipase as phospholipase A_1, A_2, C and D. When treating the ethanol-treated phospholipids (containing minor PC), phospholipase selectively catalyze hydrolization of PE and PC but not PI. More special, alkaline or acid phospholipase catalyzes hydrolization of PA and some salts produced by PE hydrolization but doesn't act on PI, PC or PE. PI products used in various fields can be obtained by this method and the purity can reach up to 99%. Lypase can be used to purify PI as well, and the purifies are 60%-70% (Deng et al., 2003).
Enzymatic method has wide application prospect as it is simpler, more convenient and environmental prospective compared with solvent method and column chromatography.

4.2.4 Other methods

Ion exchange resin may be applied to isolate PI from phospholipids mixture. The resin adsorbing PI include diethylaminoethylcellulose, diethylaminoethylagarse and quaternaryammoniumethylsephadex etc. (Deng et al., 2003).

4.3 Soybean phosphatidylethanolamine (SPE)

Solid-liquid extraction is performed using powdery soybean phospholipids and ethanol. The parameters are as follows: ratio of phospholipids to ethanol 30g/L, ethanol concentration 95%-100% (100% is optimal), extracting temperatures -15-50 degrees Celsius (-15 degrees Celsius is optimal) and extracting time 5min-11min (8min is optimal). PE content increases from 19.8% in raw material to 62.8% (An et al., 2006).
Phospholipids are dissolved in isopropanol below 65 degrees Celsius to reach a final concentration in the range of 1%-4% (w/v). The mixture is cooled to 26 degrees Celsius, allowed to rest and the precipitate is filtered and dried to obtain PE of 74.7%-79.9% pure (Ni, 1995).
Zhensheng Zhong et al. (2008) removed oil and fatty acids in powdery phospholipids with acetone first, and removed PC with repeated ethanol extraction due to PC has larger solubility in aliphatic alcohol than PE and PI, and finally enriched PE with petroleum ether extraction due to PE is soluble in ether while PI not.
The powdery soybean phospholipids are extracted repeatedly with acetone to remove oil and refined phospholipids containing 35% PE are obtained. PC is removed by repeated absolute ethanol extraction with heating and stirring and alcohol insolubles are obtained. The alcohol insolubles are extracted 3 times with petroleum ether in the 1:3-1:6 (1:4 is optimal, v/v) ratios at 30-60 degrees Celsius (30 degrees Celsius is optimal). PE obtained this way is 93.5% pure and has a yield of 91.9% (Zhong & Wei, 2008).

4.4 Soybean phosphatidylserine (SPS)

Pure PS is a white waxy solid. It's soluble in most of the nonpolar solvents containing little water, insoluble in anhydrous acetone and can be extracted from histiocyte with chloroform

and methanol. When PS is dissolved in water, most of the insoluble lipids form micell while very few form true solution. PS has one positive and two negative charges, resulting in a net negative charge. PS can be hydrolyzed by weak base into metal salts of fatty acids and a remained portion, and by strong alkali into fatty acids, serine and glycerol phosphate. PS is ready to oxidize upon exposure to the air, and the color gets darker from white to yellow and finally black. Natural PS practically isn't affected by alcohol while saturated PS can form interwoven catenulated gel with alcohol and dipalmiloyl-phosphatidylserine can interact with 5% alcohol at room temperature to form regular gel.

PC is dissolved in organic phase while the enzyme and serine are dissolved in aqueous phase. After preheating for a while, the two phases are combined, and reaction occurs at the interface under certain conditions. PS is obtained by isolating and extracting of the organic solvent phase and quantified by thin layer chromatography (TLC). The parameters are as follows: ratio of organic phase to aqueous phase 4:4 (v/v), PC concentration 75mg/ml, reacting temperature 40 degrees Celsius, pH of aqueous phase 4.0 and reacting time 12h. PS yield is 68.9% (Yang, 2010).

Blokland et al. (1999) compared the effects of bovine cortex phosphatidylserine (BCPS), SPS and egg phosphatidylserine (EPS) on cognitive competence of middle aged rats. The dosage given to lab mice was 15mg/kg.d. Changes of emotional behavior and cognitive competence in open field experiment, Morris water maze and two-dimension active avoidance experiment were observed. Arjan Blokland discovered that SPS and BCPS exhibit similar cognitive competence-improving effects which were higher than that of EPS. SPS might be a substitute for BCPS.

4.5 Preparation of phospholipids for injection

1 portion of powdery soybean phospholipids is mixed with 12 portions of distilled water and stirred to form colloidal dispersion liquid in boiling water bath before 1.8 times the weight of raw material of anhydrous sodium sulfate is added. The saturated sodium sulfate solution is discarded after blocky phospholipids are precipitated. Then 5 portions of distilled water and 0.8 portions of anhydrous sodium sulfate are used to repeat the salting out procedure. The salting-out soybean phospholipids are dried at reduced pressure and 70 degrees Celsius in water bath in vacuum drier, transferred into three-mouth bottle followed by addition of 8.7 times the weight of raw material of 95% ethanol and reflux extraction at 80 degrees Celsius in water bath with stirring for 1h. After cooling, the ethanol solution containing phospholipids is poured out and stored in refrigerator overnight to precipitate PC. The ethanol solution containing soybean phospholipids is poured out, heated to about 35 degrees Celsius in water bath before addition of activated aluminum oxide of 0.5 times the weight of raw material, stirred for 1h and filtered. The ratio of powdery phospholipids, ethanol and aluminum oxide is 1:8:0.5 (w/w/w).

The above ethanol solution is poured out followed by addition of activated carbon of 0.22 times the weight of raw material, stirring for 1h and filtration with sintered funnel. The filtrate is transferred into the distillation flask and subjected to reduced pressure distillation at 70 degrees Celsius in water bath under nitrogen to remove ethanol. Diethyl ether of 0.75 times the weight of raw material is added into the distillation flask to dissolve the dried soybean phospholipids. The diethyl ether solution is bottled and the bottle is airtight after filling in nitrogen and stored in refrigerator overnight before ultrafiltration. The diethyl ether is removed by reduced pressure evaporation at 40 degrees Celsius in water bath under nitrogen in evaporator. Anhydrous acetone is added into the glutinous soybean

phospholipids in the evaporator. The mixture is pestled and embathed for several times to remove the residual oil and moisture. Then a powdery parenteral soybean phospholipids product is produced. It exhibits the following characteristics: AV 9.9, IV 91.29, nitrogen content 1.9%, phosphorus content 4.08% and AI 99.3% (PC content is 96.7%) (Shao et al., 2000).

5. Extraction and purification of individual molecular species of soybean phospholipids

Extraction and purification of phosphatidic acid of C_{18} fatty acids

Powdery soybean phospholipids containing 20% PA are extracted with five folds (by weight) of 95% ethanol at 45 degrees Celsius for 2h with stirring at the speed of 100rpm. After centrifugation at 700xg for 10min, the supernatant is discarded. The above extraction procedure is repeated four more times until the ethanol fraction is colorless. The solid fraction is extracted with 5 folds (by weight) of methanol with a stirring speed of 100rpm for 12h at room temperature. The methanol fraction is obtained after centrifugation at 700xg for 10min. The methanol extraction procedure is repeated three times in total. The methanol fractions are combined. If the methanol is removed by evaporation, the solid residue will contain about 50% PA.

The pH of the methanol extract is adjusted to 8-9 by 1mol/L sodium hydroxide and obvious white precipitate is observed. The supernatant is obtained by centrifugation at 700×g for 5min. If the methanol is removed, the solid residue will contain about 70% PA. The pH of the supernatant is adjusted to 5-6 by 1mol/L hydrochloric acid before n-hexane of four times the volume of the methanol solution is added and mixed. The n-hexane phase is obtained after extracting for 15min and being left standing still for 2h. The n-hexane is evaporated at 45 degrees Celsius and the residue is dissolved in methanol of ten times the volume of the n-hexane phase. 60% zinc chloride solution is added into the methanol solution until no more white precipitate is formed. The precipitate is obtained by centrifugation at 700×g for 10min, washed three times with acetone which is removed by filtration and dried under nitrogen gas steam. The solid obtained is PA of C_{18} fatty acids of about 98% pure. The yield is 1/60 of the raw materials (Liu et al., 2008).

6. Applications of phospholipids

Phospholipids are widely applied in pharmaceutical field, food, feed, agriculture, daily chemical industries and other chemical industries.

Pharmaceutical field: Phospholipids exist in all of the biomembranes and play important roles in such various physiological processes as regulating of cell osmosis and membrane enzymes, transmitting of lipoids and sterols and metabolizing of cyanocobalamin, folic acid and methionine etc. Brain tissues contain 25% of phospholipids, and the metabolic abnormality of phospholipids may lead to such diseases as cancer and Alzheimer's Disease etc. High-dosage phospholipids can effectively treat neurological disorders and other diseases of nervous system. In recent years, almost 25% of the non-food application patents of phospholipids are about their applications in pharmaceutical field, especially the applications of liposomes. Phospholipids exhibit huge potential in health care products market.

Food industry: The amount of phospholipids used in food is usually 0.1%-2% of the fat in food. Phospholipids are used in margarines, shortenings, candies, soup bases, pot foods, instant foods (e.g. milk powder), bakery products (e.g. bread, cookie, dessert, biscuit and cracknel) and processed foods of meat and seafood etc. They are also used as coatings of can, soup packaging and casing of meat such as sausages etc.
Feed industry: Phospholipids are applied in animal feed such as milk replacer for calf and feed of cattle, pig, poultry, hairy animals, pets and aquatic animal (e.g. fish and crustaceans) etc. Phospholipids are the essential additives of the eel feed as they decrease the diseases of the eel and improve their growth.
Agriculture: Phospholipids can inhibit the growth of powdery mildew on cucumber, eggplant, green pepper and strawberry. The solution of 0.1% phospholipids-sodium carbonate can effectively inhibit orange green mold, cucumber powdery mildew and rice blights. Phospholipids are used as the coating components after harvesting of fruits and vegetables to improve the storage effect. Phospholipids are additives of pesticide formulae which can improve the adhesivity and permeability of pesticide and reduce their toxicity to plants.
Daily chemical industries: Phospholipids are applied in such cosmetics as moisturizer, facial cleanser, sunblocking cream, soap, bath oil, shampoo, hair care agent, shaving cream, shave clean agent, nail polish, makeup powder, blush, rouge, eye shadow, lipstick and hairspray etc. Applying of phospholipids in detergent can improve the dirt-removing power of anionic detergents.
Other chemical industries: Phospholipids are widely used in various paints, wax, shoes polish, wood preservatives, mold spray, tape coating, printing ink, ink, toner, additives of photographic materials and polyamide coating etc. as well as in papermaking and printing. They are also widely used in cement, pitch, tar shingle, surface sealant of linoleum and putty gum etc.

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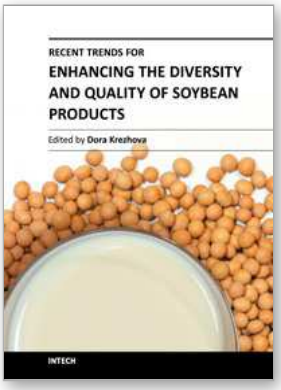

Recent Trends for Enhancing the Diversity and Quality of Soybean Products

Edited by Prof. Dora Krezhova

ISBN 978-953-307-533-4
Hard cover, 536 pages
Publisher InTech
Published online 28, October, 2011
Published in print edition October, 2011

This book presents new aspects and technologies for the applicability of soybean and soybean products in industry (human food, livestock feed, oil and biodiesel production, textile, medicine) as well as for future uses of some soybean sub-products. The contributions are organized in two sections considering soybean in aspects of food, nutrition and health and modern processing technologies. Each of the sections covers a wide range of topics. The authors are from many countries all over the world and this clearly shows that the soybean research and applications are of global significance.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Daicheng Liu and Fucui Ma (2011). Soybean Phospholipids, Recent Trends for Enhancing the Diversity and Quality of Soybean Products, Prof. Dora Krezhova (Ed.), ISBN: 978-953-307-533-4, InTech, Available from: http://www.intechopen.com/books/recent-trends-for-enhancing-the-diversity-and-quality-of-soybean-products/soybean-phospholipids

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