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Phospholipid fatty acid composition of modified soybeans and the effect of saturated fatty acid content on seed performance

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Phospholipid fatty acid composition of modified soybeans and the effect of saturated fatty acid content on seed performance

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CHAPTER 1. GENERAL INTRODUCTION

Rationale

Modification of the fatty acid composition of soybean oil to make it more competitive in various segments of the food and industrial oil markets (Hammond, 1992) has been an important objective of plant breeding and molecular genetics in recent years. Soybeans with altered fatty acid compositions have been developed through traditional plant breeding (Wilson et al., 1981) and application of chemical mutagens (Hammond and Fehr, 1975; Wilcox et al., 1984; Fehr et al., 1991a and b), and the ranges of the five major fatty acids normally found in soybean oil (palmitate, stearate, oleate, linoleate and linolenate) have been extended (Wilson, 1991). The reasons for changing the oil composition are: (1) to increase oxidative stability by reducing the polyunsaturated fatty acid percentages and/or elevating oleate percentage; (2) to improve oil nutritional value by reducing the saturate percentages; and (3) to obtain oil with desired melting characteristics by elevating the saturate percentages. Soybean oil enriched in saturated fatty acids would need less or possibly no hydrogenation for use in shortening and margarine applications. Therefore processing costs can be reduced and *trans* fatty acid formation minimized. Soybean lines with reduced linolenate and elevated oleate percentages (Hammond and Fehr, 1983; Wilson, 1984; Wilcox and Cavins, 1986; Fehr et al., 1992), soybean lines with reduced palmitate percentages (Erickson et al., 1988; Fehr et al., 1991b; Hammond and Fehr, 1983), and soybean lines with inceased palmitate and stearate percentages (Erickson et al., 1988; Fehr et al., 1991a; Hammond and Fehr, 1983) have been developed. However, there probably are limitations on the kind and extent of modifications that can be made without seriously affecting the agronomic performance of the seeds. Our lack of understanding

about these aspects of lipid biosynthesis and function can become an important barrier to producing soybean oils with economically desirable compositions. For example, soybeans with elevated stearate percentages have poor and unpredictable field germination and yield for unknown reasons (personal communication with Walter R. Fehr, Agronomy Department, Iowa State University), and these problems led us to investigate the possible causes for abnormal seed behavior.

The hypothesis of this research was that when soybean oil composition is modified, some other biologically important constituents are altered as well, and this may contribute to poor seed performance. Aside from the triglycerides (TGs), which typically make up more than 99% of refined soybean oil, soybeans also contain 0.3 to 0.6% phospholipids (PLs), which are chiefly phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) (Hui, 1996). These PLs are the major components of cell membranes that form the outer boundary of cells and also divide them into various compartments. It is important that these membrane PLs be in the proper physical state for cells to perform their physiological and structural functions, and this proper physical state requires that the PLs have the correct balance of saturated and unsaturated fatty acids, as well as a balanced PL class composition (Chapman, 1973). The arrangement of fatty acyl groups within individual PL classes can be an important factor in determining their physical characteristics and is carefully controlled (Thompson and Martin, 1984). Because the biosynthesis of TG and PLs are interrelated (Gurr, 1980; Mudd, 1980), it is expected that the modification of oil composition will cause alterations of chemical composition, physical properties, and physiological functions of the PLs.

Therefore, the objectives of this study were to determine the effect of modification of soybean oil composition on PL composition; to correlate seed performance with saturated fatty acid contents; and to examine the physical properties of the neutral and polar lipids.

Literature Review

PL biosynthesis

In plants, *de novo* fatty acid biosynthesis occurs exclusively in plastids, and palmitic, stearic, and oleic acids attached to acyl-carrier protein (ACP) are the primary products. These fatty acids are released from ACP by the action of thioesterases, which play an important roles in controlling the types and quantities of fatty acids released. The acyl residues are subsequently exported from the plastids to the cytosol and converted to acyl-CoA esters by an acyl-CoA synthetase, and these esters are used in the biosynthesis of various lipid classes (Topfer et al., 1995).

Both TG and PL are synthesized on the endoplasmic reticulum (ER) membrane starting with the acylation of *sn*-glycerol-3-phosphate with acyl-CoA from the acyl-CoA pool. This reaction is catalyzed by glycerol-3-phosphate acyltransferase and is followed by acylation of 1-acyl-sn-glycerol-3-phosphate (lysophosphatidic acid) by lysophosphatidic acid acyltransferase (LPAAT) to form phosphatidic acid (PA). This enzyme is substrate specific (Topfer et al., 1995). In rapeseed, for example, LPAAT is most efficient with C18 unsaturated fatty acids but does not incorporate erucic acid or fatty acids of medium chain length into the *sn*-2 position during TG biosynthesis. The specificities and selectivities of the glycerol acylation enzymes determine the stereospecific distribution of different acyl groups on the lipid molecules (Stymne et al., 1990). PA can also arise *via* two other pathways that are probably quantitatively less important. These are (1) the phosphorylation

of 1,2-diacyl-*sn*-glycerol by the diacylglycerol kinase and (2) the acylation of 1-acyl-*sn*-glycerol-3-phosphate arising through the reduction of 1-acyl-dihydroxyacetone-3-phosphate (Thompson and Martin, 1984). PA serves as a branch point in TG and PLs biogenesis. It is either dephosphorylated by phosphatidic acid phosphatase, yielding diacylglycerol (DG), or it is converted to CDP-DG by CTP:diacylglycerolphosphate cytidyltransferase. DGs generated by the former reaction are utilized for the synthesis of PC, PE, or TG, whereas CDP-DG is the precursor of PI and other types of PLs (Mudd, 1980; Thompson and Martin, 1984; Voet and Voet, 1995). Activated phosphate esters of choline and ethanolamine (in the forms of CDP-choline and CDP-ethanolamine) react with the *sn*-3 hydroxyl group of DG to form PC and PE. In the synthesis of PI, the active CDP-DG reacts with inositol to form the phosphodiester bond. The PLs synthesized are used for various membrane assemblies. DG is also esterified with an acyl-CoA by a diacylglycerol acyltransferase to form TG, and this is the only enzymatic step unique to TG synthesis. The TGs formed are then secreted from ER by a budding mechanism (Gurr, 1980; Huang, 1996) and oil is deposited as oil bodies.

The addition of functional groups and more double bonds to the acyl chains takes place primarily after they are esterified to various PLs. Fatty acids with double bonds in addition to the $\Delta 9$ double bond are produced by membrane-bound desaturases that use acyl groups esterified to PLs as substrate, and one of the most efficient substrates for desaturation is PC (Sperling and Heinz, 1993; Sperling et al., 1993). Polyunsaturated fatty acyl groups on PC can be exchanged with the acyl-CoA pool by phospholipase A_2 hydrolysis, and also PC esterified with polyunsaturated fatty acid may be converted to a variety of DGs by a PC-DG interconversion process (Stymne and Stobart, 1987). This

close relationship between TG and PL biosynthesis suggests that the fatty acid composition of PLs can be affected by TG genetic modification.

The distribution of fatty acyl groups in seed oil is generally believed to be 1,3-random-2-random (Litchfield, 1972). This theory states that there are two pools of fatty acids which are separately and randomly distributed to the 1,3- and 2-positions of all glycerol molecules. There is little information on PL fatty acid distribution theory, although the 1-random-2-random theory is widely accepted. As in TGs, saturated acyl groups locate primarily on the *sn*-1 position of PLs and unsaturated group are more concentrated on the *sn*-2 position. This can be explained by the specificities of the acyl transferases and the location of desaturation during PL biosynthesis.

A major approach in modifying the composition of plant storage oil is to change the degree of fatty acid saturation by altering either the activity of the desaturases or the quantities of these enzymes. Another approach is to modify the activities or quantities of the thioesterases (Topfer et al., 1995). With the rapid emergence and application of new biotechnologies, many agricultural crops with modified oil compositions have been and will be developed.

PL composition and physical properties

There has been little information available about the effect of genetic modification of soybeans on PL fatty acid composition (Mounts et al., 1996), although membrane lipid modification as an adaptive mechanism of organisms in response to environmental temperature has been extensively studied (Lynch and Thompson Jr., 1984; Lynch and Steponkus,1987; Maresca and Cossins, 1993; Fodor et al., 1995; Suutari et al., 1996). Mounts et al. (1996) reported that fatty acid composition of PLs changed with modification

of the linolenic acid and saturated fatty acid content. It is also generally believed that saturated and unsaturated fatty acids are preferentially esterified at the *sn*-1 and *sn*-2 position of the PL molecules (Olsson and Salem Jr., 1997), but it is unknown if oil modification causes unusual PL fatty acid stereospecific distributions. Both the PL overall composition and the arrangement of the fatty acids are important factors in determining PL physical and physiological characteristics (Thompson and Martin, 1984).

PLs form fluid bilayer structures under physiological conditions according to the membrane fluid mosaic model proposed by Singer and Nicholson (1972). This model states that: (1) PLs function as solvent for membrane associated proteins; (2) a portion of the membrane lipid specifically interacts with membrane protein and the physical property of these PLs is very important for protein function; (3) PLs form a permeability barrier; and (4) the membrane bilayer can undergo thermally induced phase transition, which is a transition from a well-ordered solid gel state to a disordered fluid-like liquid-crystalline state that is essential for membrane biological function. The membrane phase transition has been intensively studied, since the local fluidity may regulate membrane-mediated processes (Melchior and Steim, 1976).

The membrane transition temperature depends primarily on the chain length and degree of unsaturation of the acyl chain, and is only secondarily dependent on the polar head group composition (McElhaney, 1994). The transition temperature varies modestly with increases in chain length but can vary markedly with changes in degree of unsaturation of the lipid hydrocarbon chain. The greater sensitivity of membrane phase transition temperature to the chain unsaturation, rather than to chain length or polar head group structure, is probably the reason why living organisms normally alter the relative proportion of saturated and unsaturated fatty acyl groups in their membrane lipids in response to

changes in environmental temperature, rather than altering chain length or head group distribution (Lewis and McElhaney, 1992; Kawaguchi and Seyama, 1984; Fulco, 1984; Russell, 1984). Numerous studies have firmly established that there are two extreme unphysiological states of the organization of the lipid bilayer in a biological membrane that can inhibit or abolish cell growth and certain membrane functions (McElhaney, 1982; McElhaney, 1985; McElhaney, 1986). The first is a 'hyper-rigid' state that arises under low environmental temperatures. The second state is 'hyper-fluid' state in which the fluidity of the liquid crystalline bilayer becomes too great because of a high environmental temperature. Membranes in these states cannot function as effective permeability barriers, and the activities of membrane-associated enzymes and transport proteins can be reduced. Quinn and Williams (1978) suggested that the major differences between chilling-sensitive and chilling-resistant plants may lie in the fluidity of their mitochondria and chloroplast membranes. The greater fluidity of the membranes of the organelles of chilling-resistant plants allow them to perform their metabolic functions at lower temperatures and thus prevent chilling injury. The molecular basis of chilling sensitivity in plants is related to the nature of the acyl residues associated with the membrane lipids (Quinn, 1981). It has been proposed that the fluidity of the liquid-crystalline bilayer must be maintained within quite narrow range for optimal cell function and growth (Chin and Goldstein, 1985; van Blitterswijk, 1985; Butterfield, 1985; Gould and Ginsberg, 1985; Gordon and Mobley, 1985). Therefore, when assessing the temperature adaptation of the lipids in biological membranes, physical techniques that can monitor both phase transition and lipid fluidity may be desirable.

Many physical techniques, either non-perturbing or potentially perturbing, can be used to monitor the membrane phase transition and lipid fluidity. X-ray diffraction and

nuclear magnetic resonance (NMR) are non-perturbing techniques that are usually used to monitor phase transition. Electron-spin resonance (ESR) is a perturbing technique that measures both phase transition and lipid fluidity in the fluid state. Fluorescence polarization (FP) is the most popular technique for measuring fluidity by using an extrinsic probe. Fourier transform infrared (FTIR) spectroscopy is non-perturbing and can be used to assess lipid phase state and fluidity. Differential scanning calorimetry (DSC), which measures the heat absorbed (or released) by a sample as it undergoes endothermic (or exothermic) phase transition, is particularly useful (Cullis and Hope, 1991). DSC instruments provide an accurate, quick, relatively sensitive and inexpensive determination of lipid phase transition, but this technique does not measure lipid fluidity. McElhaney (1994) suggests that DSC and NMR are the methods of choice for studies of lipid phase transition and fluidity in the liquid-crystalline state, respectively.

Viability and vigor of modified soybeans

Soybean seeds have evolved to their current function as a survival tissue. In this evolution, the seeds have adapted to a particular embryo and reserve oil composition. When the reserve composition is modified without any regard for the consequences to the embryo, it would not be surprising to find significant differences in seed physiological properties for some modifications. There is no documented information about the effect of soybean oil composition modification on seed performance, although the general observation is that modification does not critically affect seed agronomic behavior, except when the stearic acid percentage is modified (personal communication with Dr. W.R. Fehr, Agronomy Department, Iowa State University).

The standard germination test is the most widely accepted test for estimating seed viability (Dornbos, 1988). Germination is defined as the emergence and development of essential structures from the seed embryo that are needed to produce a normal plant under favorable conditions (AOSA, 1986). But the germination test is an inadequate predictor of field emergence because field conditions are seldom as optimal as standard test conditions and in the standard test, seedlings are classified as either germinable or non-germinable after a seven day period without regard to the progressive nature of seedling growth or seed deterioration (Dornbos, 1988). Tests of seedling vigor were developed to complement the viability test. Vigor is defined in the Vigor Testing Handbook (AOSA, 1983) as those seed properties that determine the potential for rapid, uniform emergence and development of normal seedlings. Two of the many accepted vigor tests are: (1) the seedling growth rate, which evaluates the ability of seed to convert its storage reserves into seedling axis dry weight; and (2) the conductivity test, which is a measurement of electrolyte leakage from plant tissue and is an indication of the cell membrane integrity of the seed. Cell membranes lose their integrity as seeds dry at maturity, but during imbibition this membrane integrity is re-established. Vigorous seed re-establish membranes at a faster rate with less leakage than less vigorous seed. Conductivity has been negatively correlated with seed viability and vigor, and has implications in commercial seed testing (McKersie and Senaratna, 1983).

PL molecular species composition analysis

Molecular species composition change is a very important adaptation mechanism for microorganisms and causes significant change in membrane physical properties due to acyl chain rearrangement in PL molecules without overall compositional change (Lynch and Thompson Jr., 1984). Mounts et al. (1996) showed some evidence that soybean oil

modification caused considerable change in molecular species composition, but more detailed quantitative evaluation is needed to determine how genetic modifications affect PL molecular species composition.

Traditionally, two types of analytical approaches, high-performance liquid chromatography (HPLC) and gas chromatography (GC), are used for PL partial molecular species analysis (Olsson and Salem Jr., 1997). PLs are hydrolyzed with phospholipase C to obtain 1,2-diacyl-sn-glycerols (DG), which are then converted to either HPLC- or GC-amenable derivatives. In HPLC applications, conversion of DG to UV absorbing derivatives, for instance benzoate derivatives, has been used frequently. In GC applications, the DG may be converted to corresponding monoacetyldiacylglycerols, trimethylsilyl (TMS) ethers or *tert*-butyldimethylsilyl (t-BDMS) ethers. A general drawback of these approaches is that the derivatizations may subject the lipid to the danger of acyl chain rearrangement and migration. Direct analysis of the underivatized, i.e. 'intact' PLs by reversed-phase HPLC is the best option for PL fractionation, but the major difficulty is associated with effluent detection. UV detectors are not suitable for lipid detection because the degree of unsaturation of the analyte has a notable impact on signal intensity. Refractive index detectors are less versatile than UV and has many limitations. Christie and Hunter (1985) used the evaporative light scattering detection (ELSD) principle for PC molecular species analysis, and since then many other authors have reported lipid analysis using this powerful mass detector. Because the detector response to sample size has been described as linear, sigmoidal or exponential (Christie, 1992), the detector response should be tested and the data need to be corrected for an accurate quantitative analysis.

Dissertation Organization

This dissertation contains a general introduction, including a research rationale and a literature review, followed by four research papers and a general conclusion. The papers are in the required corresponding journal formats.

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CHAPTER 2. PHOSPHOLIPID FATTY ACID COMPOSITION AND STEREOSPECIFIC DISTRIBUTION OF SOYBEANS WITH A WIDE RANGE OF FATTY ACID COMPOSITIONS

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Abstract

Phospholipid (PL) fatty acid composition and stereospecific distribution of 25 genetically modified soybean lines having a wide range of compositions were determined by gas chromatography and phospholipase A_2 hydrolysis. PL contained an average of 55.3% phosphatidylcholine, 26.3% phosphatidylethanolamine and 18.4% phosphatidylinositol, PL class proportions were affected by changes in overall fatty acid composition. PL fatty acid composition changed with oil fatty acid modification, especially for palmitate, stearate and linolenate. Stereospecific analysis showed that saturated fatty acids were primarily located at the *sn*-1 position of all PLs, and changes of the saturates in PLs were largely reflected on this position. Oleate was distributed relatively equally between the *sn*-1 and *sn*-2 positions. Linoleate was much more concentrated on *sn*-2 than on *sn*-1 position for all PLs. Linolenate was distributed relatively equally at low concentration but preferred the sn-2 position at high concentration.

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Key words: Fatty acid stereospecific distribution, gas chromatography, genetic modification of soybeans, phospholipase A_2, phospholipid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, thin-layer chromatography.

Introduction

Modification of the fatty acid composition of soybean oil to make it more competitive in various segments of the food and industrial oil markets (1) has been an important objective of plant breeding and molecular genetics in recent years. Altered fatty acid compositions have been developed through traditional plant breeding (2) and application of chemical mutagens (3-6) that have extended the range of the five major fatty acids found normally in soybean oil (palmitate, stearate, oleate, linoleate and linolenate). Aside from the triglycerides (TGs), which typically make up more than 99% of refined soybean oil, soybeans also contain 0.3 to 0.6% phospholipids (PLs), with phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) being the major classes (7). These PLs are the major components of the cell membranes that form the outer boundary of the cell and divide it into various compartments. It is important that these membrane PLs be in the proper physical state for cells to perform their metabolic tasks, and this proper physical state requires that the PLs have the correct balance of saturated and unsaturated fatty acids, as well as a balanced PL class composition (8). The arrangement of fatty acyl groups within individual PL classes can be an important factor in determining their physical characteristics, and is carefully controlled (9).

PL compositional changes in the membranes of organisms in response to environmental temperature have been studied extensively (10,11). Soybeans produced under drought and high-temperature conditions contained PLs with altered fatty acid

compositions that affect the ability of the seed to maintain optimum rates of metabolism and germination (12). But information about the effect of genetic modification of soybean on PL fatty acid composition is very limited. Mounts et al. (13) reported a study on PLs, tocopherols and sterols in several soybeans with low linolenate and increased saturate percentages. They concluded that there was little impact of fatty acid composition on the proportions of the PL classes, but considerable change occurred in the molecular species present in each class.

Do soybeans with fatty acid composition modified far from its typical range have PLs that are altered in ways that may be detrimental to the plant? In this study, soybean lines with the widest possible ranges of the five major fatty acids were analyzed for PL composition and stereospecific distribution to determine the effect of alteration in the overall fatty acid composition on the composition and distribution of PLs.

Experimental Procedures

Materials. Commercial soybean cultivars and experimental soybean lines were provided by the Agronomy Department at Iowa State University. PL and lysoPL standards (from soybean) and snake venom from *Crotalus adamentus* containing phospholipase A_2 were obtained from Sigma Chemical Co. (St. Louis, MO). All organic solvents were reagent grade.

Lipid extraction and class separation. Approximately 10 g seed (fresh wt, about 9% moisture) was ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) equipped with a 20-mesh delivering tube. Duplicate samples of 2.00 g ground beans were extracted with 30 ml chloroform:methanol (v:v, 2:1) for 1 hr with stirring. The sample was filtered and washed with additional solvent, and 0.75% KCl equal to 20% of the final total volume was

added to the filtrate. The lower chloroform phase was collected, and the solvent was removed with a rotary evaporator at 40°C. Total crude lipid was measured gravimetrically. Neutral and polar lipid class separation was achieved by solid phase extraction by a modification of the method of Mounts et al. (13). A 900-mg silica cartrige (Alltech Associate, Inc., Deerfield, IL.) was loaded with about 0.4 g of crude lipid dissolved in chloroform. Neutral lipid (TG) was eluted with 30 ml chloroform and sampled for fatty acid composition analysis by GC; polar lipids were sequentially eluted with 10 ml chloroform:methanol (v:v, 1:1), 20 ml methanol and 15 ml chloroform:methanol:water (v:v:v, 1:2:0.8). Phase separation of the last elution was obtained by adding 7.6 ml chloroform and 3.0 ml water, and the lower chloroform layer was combined with the other two elutions. Solvent was removed by a rotary evaporator, and the polar lipids were redissolved in 0.5 ml chloroform:methanol (v:v, 8:2).

PL class separation. To separate the major PL classes, 0.15 ml of the total polar lipid solution was streaked on a 20 x 20 cm, 500-μ Adsorbsil-plus1 preparative plate (Alltech), and the plate was developed with chloroform:methanol:acetic acid:water (v:v:v:v,100:45:5:2). Bands were visualized by spraying with 0.1% 2',7'-dichlorofluorescein (Sigma) in methanol and viewing under UV light. PC, PE, and PI bands (Rfs were 0.28, 0.70 and 0.56, respectively) were identified by comparison with standards. The bands were scraped from the plate and extracted 5 times with 15 ml chloroform:methanol:water (v:v:v, 1:2:0.8) (14). Phase separation was performed as already described, and the chloroform layer was evaporated under nitrogen. Next, 1 ml of freshly distilled ethyl ether was added to redissolve the PLs.

PL quantification and stereospecific analysis. From the 1-ml ether solution of PLs, 0.2 ml was taken for PL fatty acid analysis and quantification. The remaining 0.8 ml

was used for the stereospecific distribution analysis. A modified Robertson and Lands procedure (15) was used for PL *sn*-2 position fatty acid hydrolysis. The PL samples were shaken gently overnight at 37°C with 0.2 ml of 5 mg/ml snake venom in a 50:50 mixture of 0.1 M borate buffer at pH 7.0 and 0.01 M $CaCl_2$. The hydrolyzed mixture was dried under nitrogen and suspended in 0.2 ml chloroform:methanol (v:v, 8:2). The *sn*-2 position fatty acid and lyso PL were separated by TLC using the same system used for PL class separation. The silica plate was carefully examined for any unhydrolyzed PL bands. The hydrolysis should be complete to ensure accurate evaluation of fatty acid stereospecific distribution. Rf values for free fatty acid, lyso PC, lyso PE and lyso PI were 0.90, 0.08, 0.34 and 0.19, respectively. Free fatty acid and lyso PL bands were scraped from the plate, and the methyl ester conversion reaction was performed directly on silica.

FAME (fatty acid methyl ester) preparation and GC. For PL fatty acid determination and quantification, a 0.2-ml aliquot of PL was dried under nitrogen and reacted with 0.3 ml of 1.0 M sodium methoxide in methanol at ambient temperature for 40-60 min with occasional shaking (16). Methyl heptadecanate (Sigma) was used as internal standard and added to the reaction mixture before the termination of the reaction. Water was added to stop the reaction, and 0.3 ml hexane was used to extract the FAME. For transesterification of lyso PL on silica, a similar procedure was applied, but without adding internal standard, and 0.5 ml sodium methoxide was used. To esterify free fatty acid on silica (17), 0.5 ml of 2% sulfuric acid in methanol was added, and the mixture was reacted at 80°C for 1 hr. The FAMEs (1 μl) were analyzed with a Hewlett-Packard (HP) (Avondale, PA) 5890A gas chromatograph equipped with a flame ionization detector and capillary DB-23 (15-m length, 0.25-mm id., and 0.25-μm film thickness) fused column from J & W Scientific (Deerfield, IL). Oven temperature was 220°C, inlet and detector temperature were

250°C; split ratio was 10:1. Theoretical correction factors were calculated (18) and applied to correct the FAME weight percentages. Mole percentages were calculated and reported.

Results and Discussion

PL content. Table 1 shows the typical soybean fatty acid composition (19) and the composition range selected for this study. Table 2 presents the average percentage of the amounts and relative proportions of the PLs. The extraction recoveries for PC, PE and PI from the scraped silica were 94.2, 99.6 and 98.8%, respectively, when known amounts of the standards were applied to TLC plate which was developed in the solvent system. A total of 25 soybean samples was analyzed. On average, the soybeans contained 23.7% crude lipid, and the crude lipid contained 3.7% total PL. Of the total PL, PC accounted for 55.3%, PE 26.3% and PI 18.4%. PL reportedly makes up 1.5 to 5.0% of the crude hexane extractables from soybean seed and contains 35 to 46% PC, 25 to 27% PE and 13 to 18% PI (12). Soybean PL also contains a minor amount of phosphatidylserine (PS), phosphatidic acid (PA) and lyso PC (7). The presence of PA and lyso PC usually has been associated with active phospholipase during seed storage (7,13) or laboratory sample manipulation.

Crude lipid percentage, amount of PC, PE, PI and total PL (mg/g bean), relative percentages of individual PL classes, and PL percentage in crude lipid were correlated with TG fatty acid percentages to explore the effect of oil composition on the quantity of PLs. The results are summarized in Table 3. These data suggested that soybean oil percentage decreased with increased levels of total saturate ($r = -0.51$), palmitate ($r = -0.60$), and linolenate ($r = -0.56$), and increased with increased levels of oleate ($r = 0.57$) and linoleate ($r = 0.46$). In contrast, the percentage of total PL in total lipid increased with increased

levels of total saturate (r = 0.61), palmitate (r = 0.62) and linolenate (r = 0.39), and decreased with increses in oleate (r = -0.49) and linoleate (r = -0.59). Total PLs and oil contents were not correlated (r = -0.06, not presented in Table 3). When oil content increases, either the size or the number of the oil bodies should increase so that the PL half membrane surrounding the oil body should also increase. But the other organell membranes also can be coextracted with oil and may mask the effect of oil body PL.

In general, the quantity of total PL did not correlate with any of the fatty acids ($p \geq 0.05$), and neither did the amount of individual PLs, except for PE, which was positively correlated with total saturate and palmitate, and negatively with linoleate. PL class compositions (percent of each PL class relative to total) were also correlated with various TG fatty acids. There were significant positive correlations of % PE with total saturates and palmitate and a negative correlation with linoleate. There also was a negative correlation between %PI and palmitate. These results suggest that fatty acid composition of soybean affects PL class composition, in contrast to Mounts et al. (13), who reported that PL class composition was unaffected by soybean genetic modification.

Relationship of PL and TG fatty acid composition. Fig.1 and 2 show the effect of changes in TG fatty ester percentage on PL fatty ester composition. Table 4 summarizes the slopes and regression coefficients (R^2) of these linear plots. In general, these plots show considerable scatter but they illustrate a number of important relations. PI had greater palmitate and stearate percentages than did PC and PE. PC had the lowest palmitate percentage, whereas PE had the lowest stearate percentage. With the increase of TG palmitate and stearate, all three PLs showed corresponding increases in these saturated esters. The slope of the PI plot was greater than those for PC and PE, suggesting that PI was the most sensitive PL to saturated fatty acid alteration. Note that for the PI palmitate

plot, some points were well above and below the line. The group of points above the line had typical TG stearate level, whereas the group below the line had elevated TG stearate. Similarly, for the PI stearate plot, the group of points above the line had decreased TG palmitate, and the group of points below the line had elevated TG palmitate. The PC and PE plots also showed similar patterns of deviation, but less strongly than those of the PI plots. These deviations suggest that the presence of either of these saturates in PLs suppresses the incorporation of the other.

The unsaturated fatty ester percentages of all PLs also were positively correlated with their TG percentages (Fig. 2 and Table 4), but these plots again showed considerable scatter. In the oleate plot, the data points well above the regression lines had either very low percentages of palmitate and stearate or very low percentage linolenate. Generally, PC, PE and PI contained similar percentages of oleate and linolenate. PC and PE contained much higher levels of linoleate than PI. The slopes of oleate and linoleate plots were smaller than those for the saturated esters and linolenate. All the slopes in Table 4 are less than one indicating that PL acyl group percentages change less than those for TG as acyl percentages vary. These results are consistent with those of Mounts et al. (13).

PL fatty acid stereospecific distribution. Fig. 3 A,B and Fig. 4 A,B,C show plots for PC, PE and PI of saturated and unsaturated fatty ester percentages on the *sn*-1 and *sn*-2 positions as the total percentage of each fatty ester in a PL class varies. Table 5 presents a summary of the linear regressions fitted to these plots. Palmitate and stearate were predominantly located at the *sn*-1 position of all PLs. The changes of palmitate in PC, PE and PI were almost exclusively reflected on the *sn*-1 position. The changes of palmitate in *sn*-2 position were not statistically significant from zero for PC ($p = 0.14$) and PE ($p = 0.12$) at the 5% probability level, and barely significant ($p = 0.04$) in PI. The slopes of *sn*-1 and

sn-2 regression lines theoretically should sum to 2.00 (20) and were 1.89 for PC, 2.15 for PE, and 2.01 for PI. The changes of stearate percentage in PC, PE and PI were reflected both on *sn*-1 and *sn*-2 positions, but the *sn*-1 position had a much greater change, as indicated by larger slopes. Although, statistically, the change at *sn*-2 position was significant ($p < 0.001$), the slopes were very small and comparable to the palmitate *sn*-2 position slopes. The slope sums were 2.00, 1.87 and 1.99 for PC, PE and PI, respectively. Therefore, saturated fatty acids primarily distributed at the *sn*-1 position of all PLs, and this position accounted for the PL compositional change.

It is well documented that saturated fatty acids predominantly occupy the *sn*-1 and *sn*-3 positions of soybean TG, and these two positions reflected the saturated fatty acid change in oil (21-23). Harper (23) used soybean lines that had fatty acid composition ranges similar to those used in this study to analyze acyl group stereospecific distribution in TG. Similar graphs and linear regressions were made. Harper found that there was little saturate at the *sn*-2 position, and that the slopes of this position for palmitate and stearate were 0.04 and 0.08, as compared with the sums of *sn*-1 and *sn*-3 slopes of 2.94 and 2.91. Thus, the *sn*-2 position palmitate and stearate in PLs had greater slopes than in TG.

Unsaturated fatty esters had a different distribution profile than those of the saturates. At low percentages of oleate in PLs, oleate was equally distributed between the *sn*-1 and *sn*-2 positions. With an increase of oleate in the PLs, more occupied the *sn*-2 position. Linoleate was much more concentrated on the *sn*-2 position than on the *sn*-1 position for all three PLs, and for all three PLs, the *sn*-1 slope was greater than the *sn*-2 slope. For all PLs, linolenate was distributed relatively equally at both positions at low concentration, but as linolenate in PLs increased the *sn*-2 position accumulated significantly more linolenate than the *sn*-1. This result again showed a great similarity to Harper's

unsaturate distribution in TG. The resemblance of sterespecific distribution of PLs and TG may be explained by their interrelated biosynthesis processes (24,25).

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Table 1. Typical soybean fatty acid composition and the composition range examined in this study

	Palmitate	Stearate	Oleate	Linoleate	Linolenate
Typical	11	4	24	54	7
Range	3.1-33.3	2.4-24.2	7.8-35.3	35.3-68.3	2.7-16.3

Table 2. Average soybean crude lipid and PL compositions and their standard deviations (S.D.)

	% Lipid[a]	PC[b]	PC%[c]	PE[b]	PE%[c]	PI[b]	PI%[c]	Tot.PL[b]	%Tot.PL[d]
Average	23.7	4.7	55.3	2.3	26.3	1.6	18.4	8.5	3.7
S.D.	3.8	1.4	3.5	0.8	2.8	0.5	2.5	2.4	1.2

[a] crude lipid in the bean

[b] mg /g bean

[c] individual PL relative to total PL

[d] total PL in crude lipid

Table 3. Correlation among PLs and crude lipid contents and TG fatty acid composition

		% Lipid[c]	PC[a]	PC%[c]	PE[a]	PE%[c]	PI[a]	PI%[c]	Tot.PL[a]	% Tot.PL[d]
Tot. Sat.	r	-0.51	0.40	-0.18	0.48	0.48	0.26	-0.30	0.42	0.61
	prob[b]	0.01	0.06	0.43	0.02	0.02	0.23	0.17	0.05	0.00
Palmitate	r	-0.60	0.32	-0.13	0.50	0.61	0.10	-0.52	0.35	0.62
	prob	0.00	0.14	0.56	0.02	0.00	0.66	0.01	0.10	0.00
Stearate	r	-0.06	0.20	-0.10	0.13	0.01	0.25	0.13	0.20	0.18
	prob	0.78	0.35	0.65	0.56	0.96	0.25	0.55	0.36	0.41
oleate	r	0.57	-0.30	-0.18	-0.26	-0.09	-0.10	0.35	-0.27	-0.49
	prob	0.00	0.16	0.42	0.23	0.67	0.64	0.10	0.22	0.02
linoleate	r	0.46	-0.37	0.29	-0.49	-0.55	-0.28	0.22	-0.41	-0.59
	prob	0.02	0.08	0.18	0.02	0.01	0.20	0.31	0.05	0.00
linolenate	r	-0.56	0.19	0.21	0.13	-0.04	0.02	-0.24	0.15	0.39
	prob	0.01	0.39	0.35	0.55	0.86	0.93	0.27	0.50	0.07

[a] mg /g bean

[b] probability of r = 0

[c] crude lipid in bean

[d] total PL in crude lipid

[e] individual PL relative to total PL

Table 4. Slopes and R^2 of the linear regressions of the percentage of various fatty esters in each PL class *versus* the percentage of the fatty ester in TG

		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
PC	slope	0.52	0.46	0.14	0.33	0.53
	R^2	0.88	0.88	0.07(0.20)*	0.34	0.57
PE	slope	0.49	0.28	0.17	0.18	0.46
	R^2	0.80	0.67	0.11(0.10)	0.20(0.02)	0.47
PI	slope	0.74	0.62	0.26	0.27	0.57
	R^2	0.61	0.62	0.35	0.57	0.70

* Numbers in ( ) indicate P value of the R^2 being equal to zero, R^2 without ( ) have $P < 0.001$

Table 5. Slopes and R^2 of linear regressions for stereospecific plots of fatty esters in PC, PE and PI

			$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
PC	sn-1	slope	1.74	1.67	0.88	1.21	0.59
		R^2	0.92	0.97	0.87	0.76	0.74
	sn-2	slope	0.15	0.33	1.11	0.59	1.34
		R^2	0.09(0.14)*	0.52	0.96	0.36	0.89
		slope sum	1.89	2.00	1.99	1.80	1.93
PE	sn-1	slope	1.96	1.72	0.78	0.97	0.63
		R^2	0.94	0.89	0.86	0.42	0.68
	sn-2	slope	0.19	0.15	1.14	0.90	1.19
		R^2	0.1(0.12)	0.46	0.94	0.41	0.84
		slope sum	2.15	1.87	1.92	1.87	1.82
PI	sn-1	slope	1.86	1.77	0.86	1.05	0.11
		R^2	0.97	0.95	0.61	0.57	0.21(0.02)
	sn-2	slope	0.15	0.22	1.08	0.64	1.76
		R^2	0.17(0.04)	0.73	0.85	0.15(0.05)	0.92
		slope sum	2.01	1.99	1.94	1.69	1.87

* Numbers in ( ) indicate P value of the R^2 being equal to zero, R^2 without ( ) have $P < 0.001$.

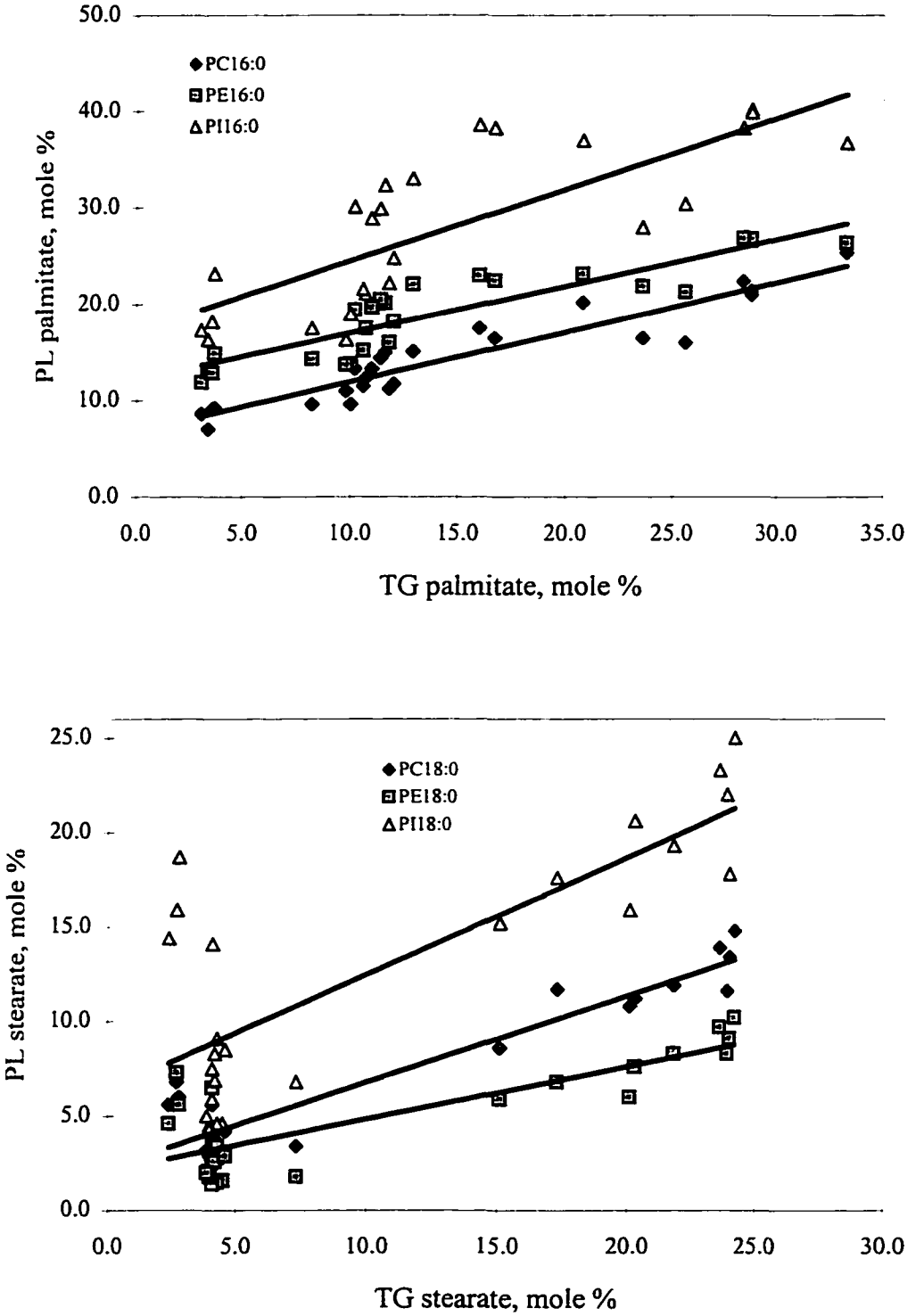


Figure 1. PL saturated fatty acids as affected by TG saturated fatty acids

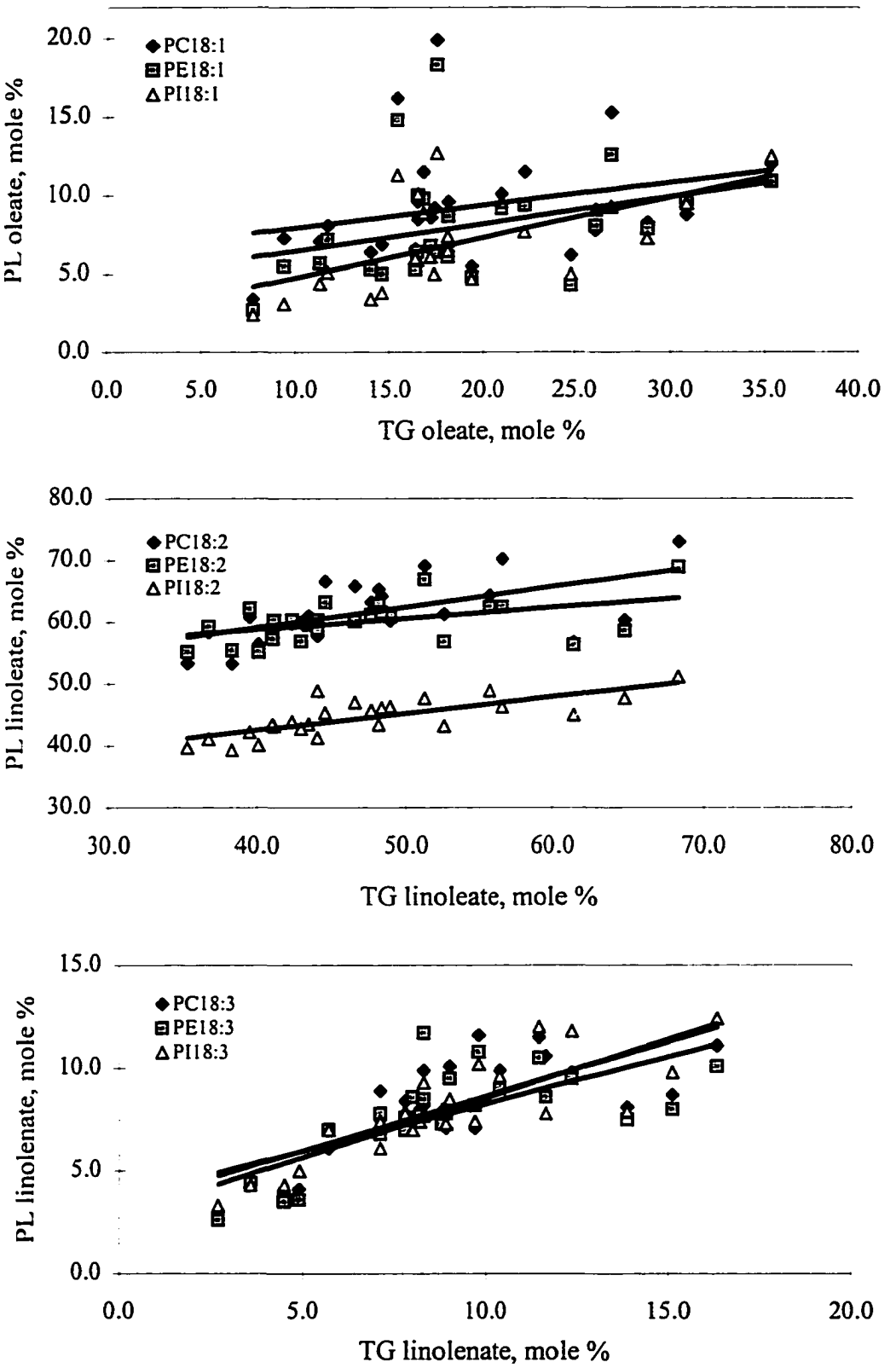
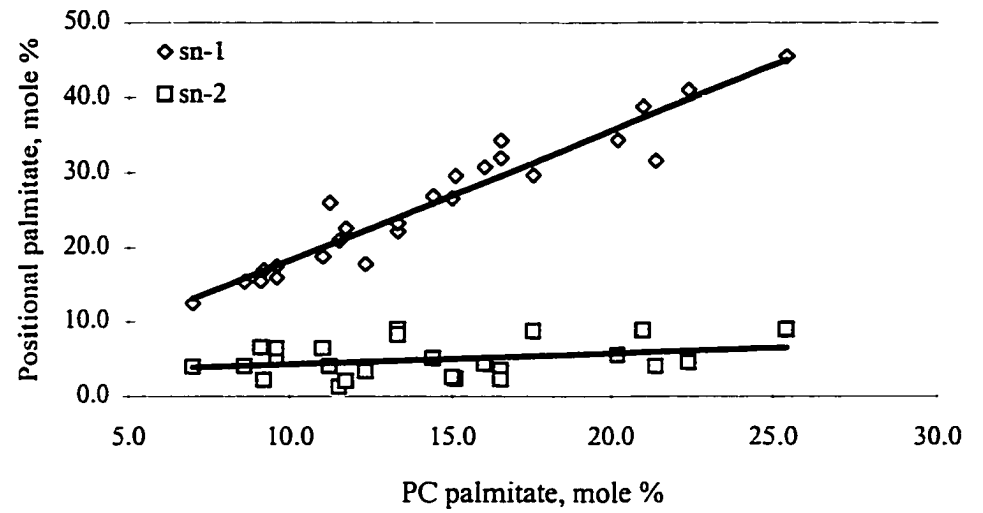
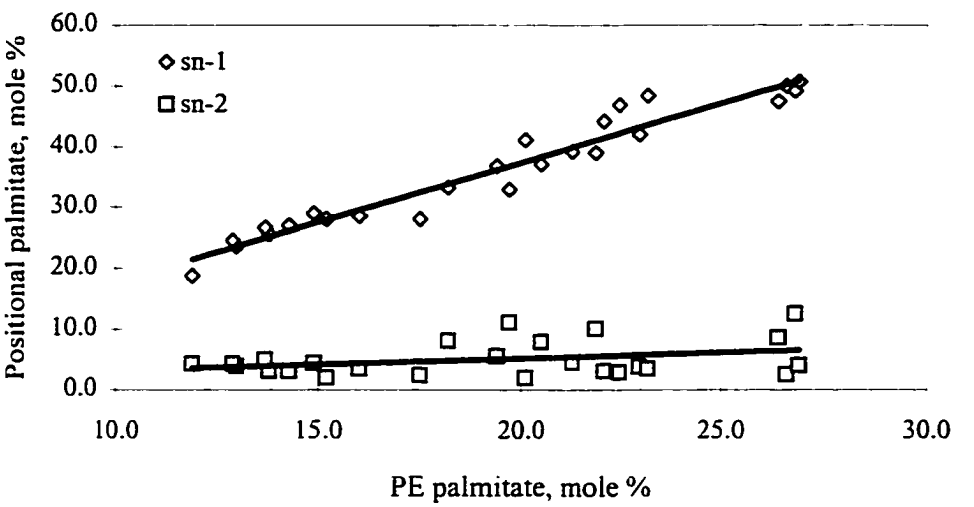


Figure 2. PL unsaturated fatty acids as affected by TG unsaturated fatty acids





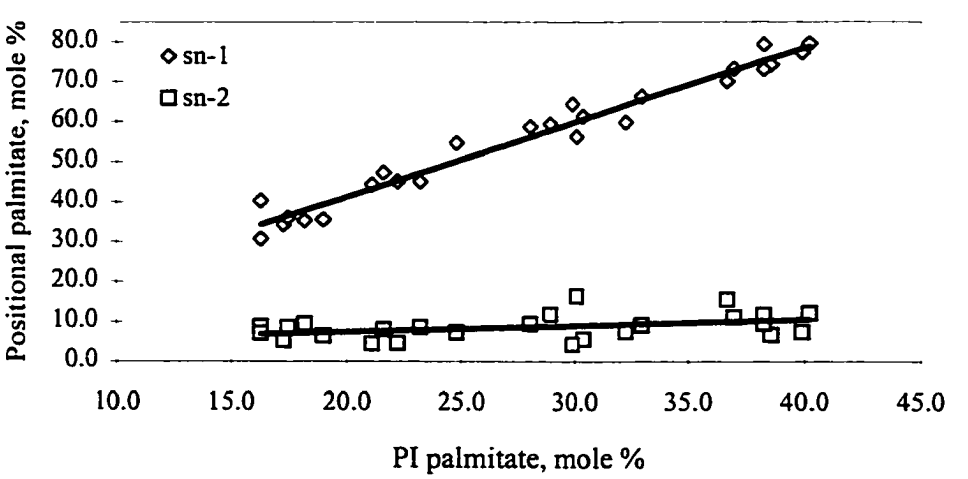
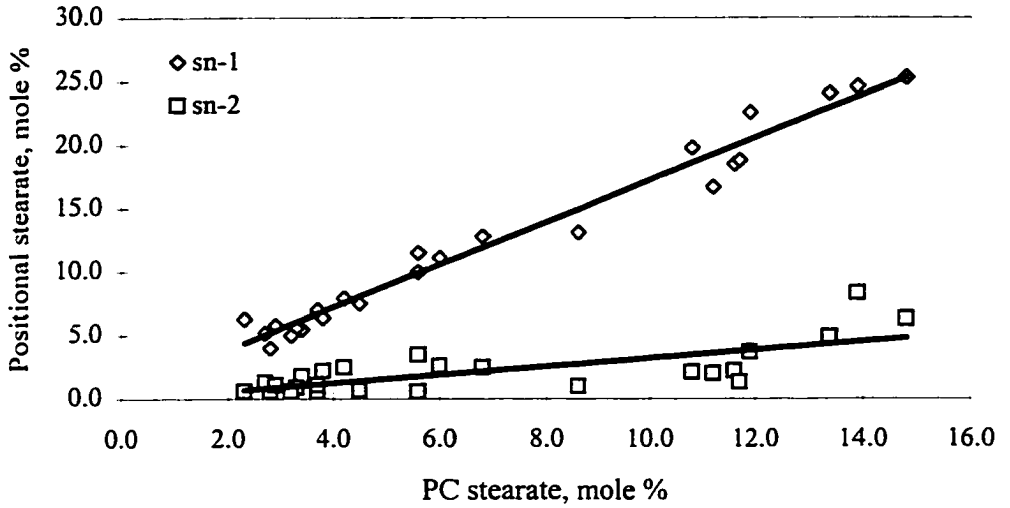
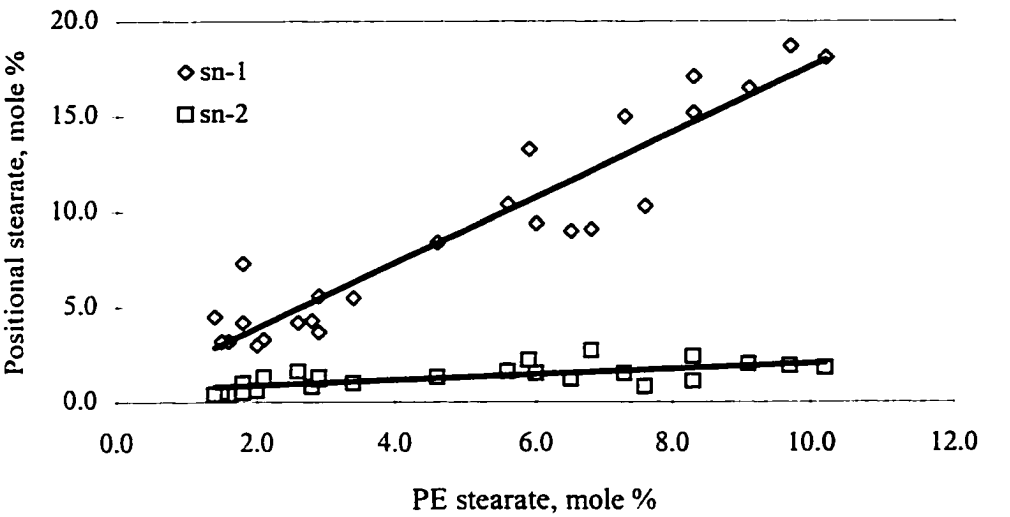


Figure 3A. PL palmitate stereospecific distribution





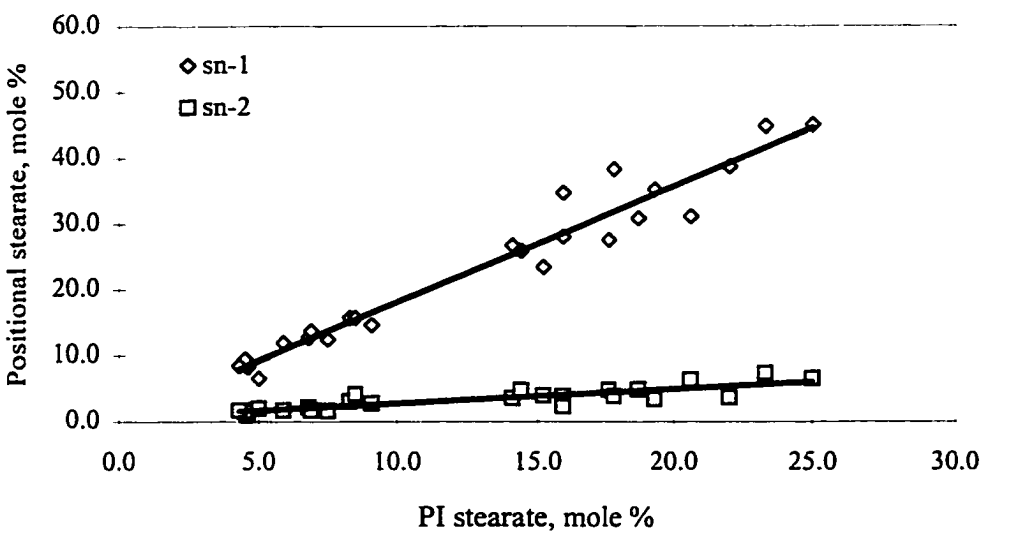
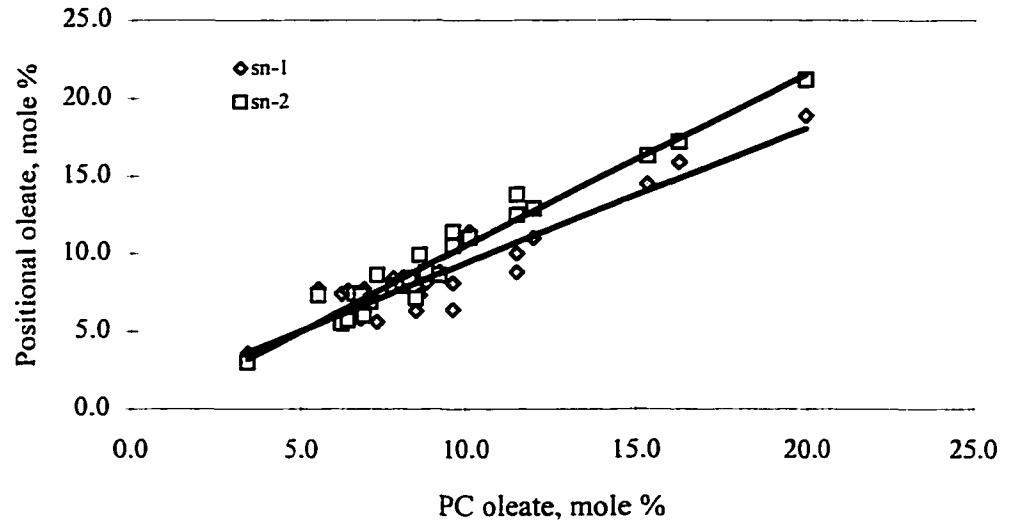
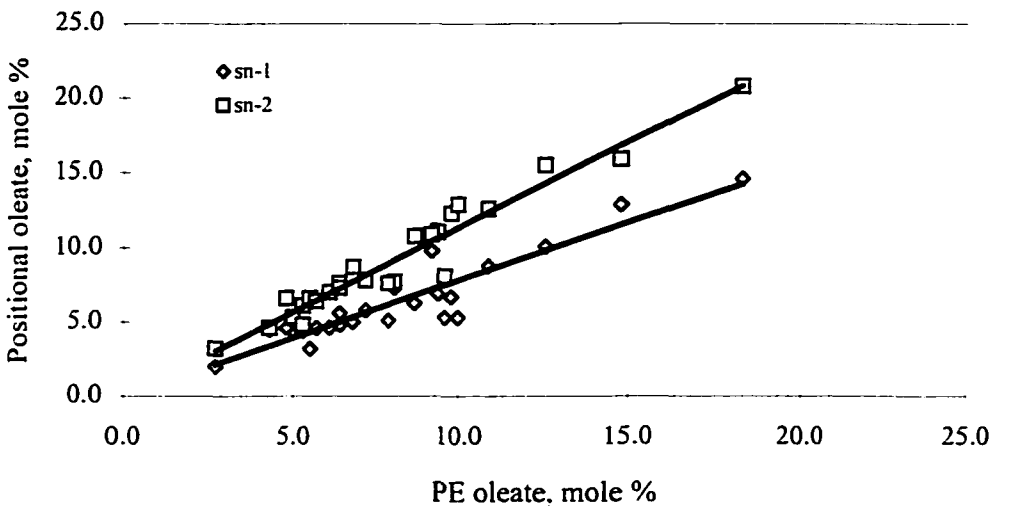


Figure 3B. PL stearate stereospecific distribution





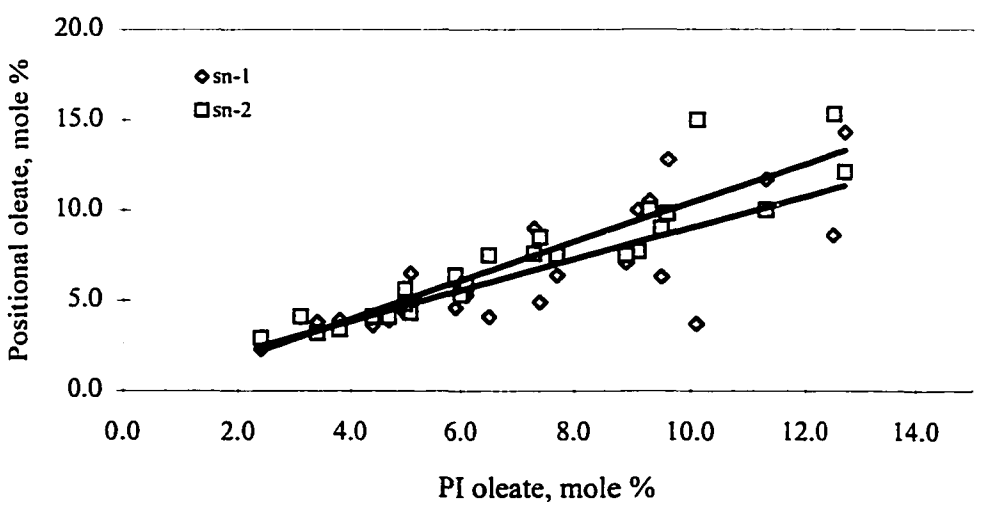


Figure 4A. PL oleate stereospecific distribution

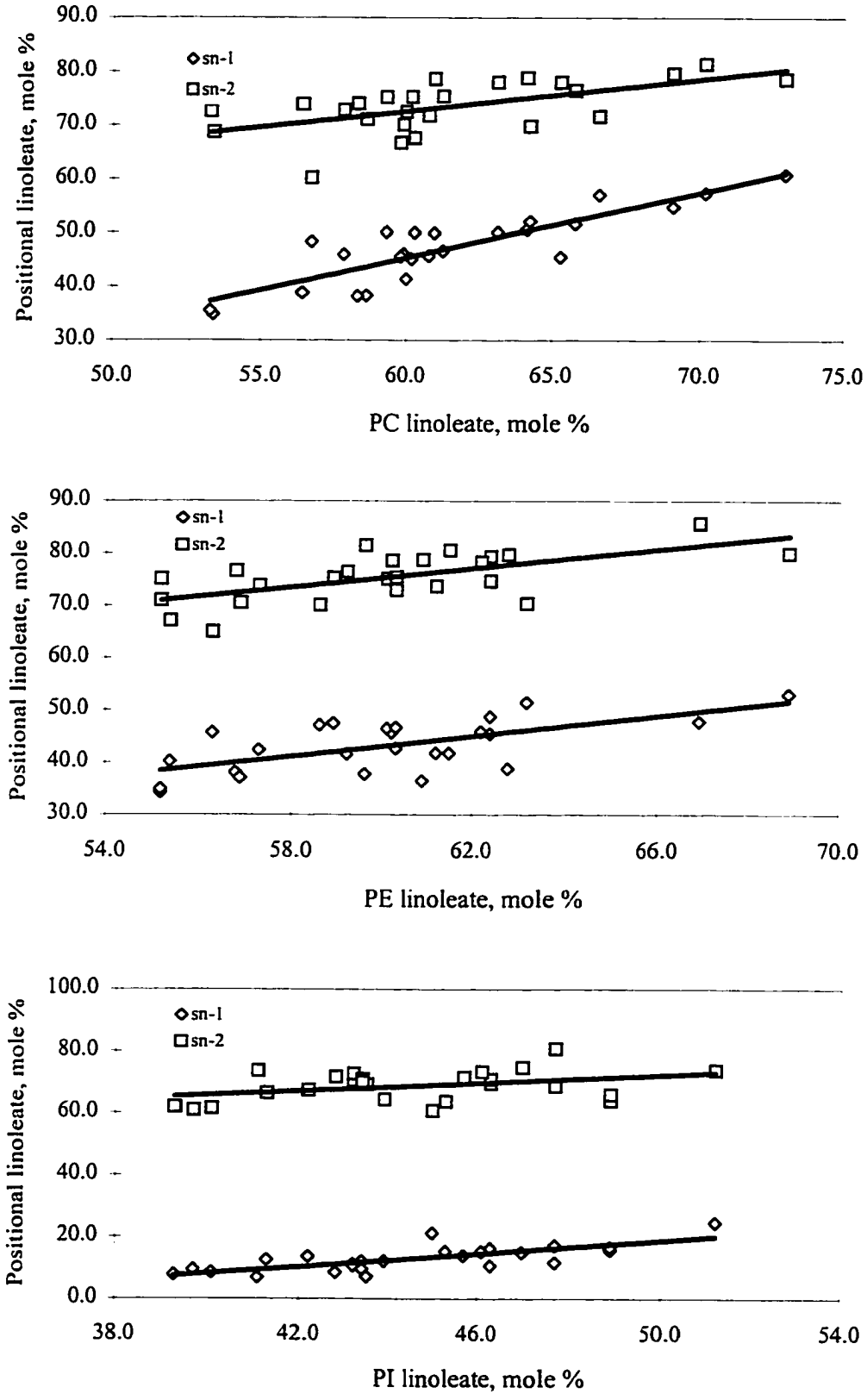


Figure 4B. PL linoleate stereospecific distribution

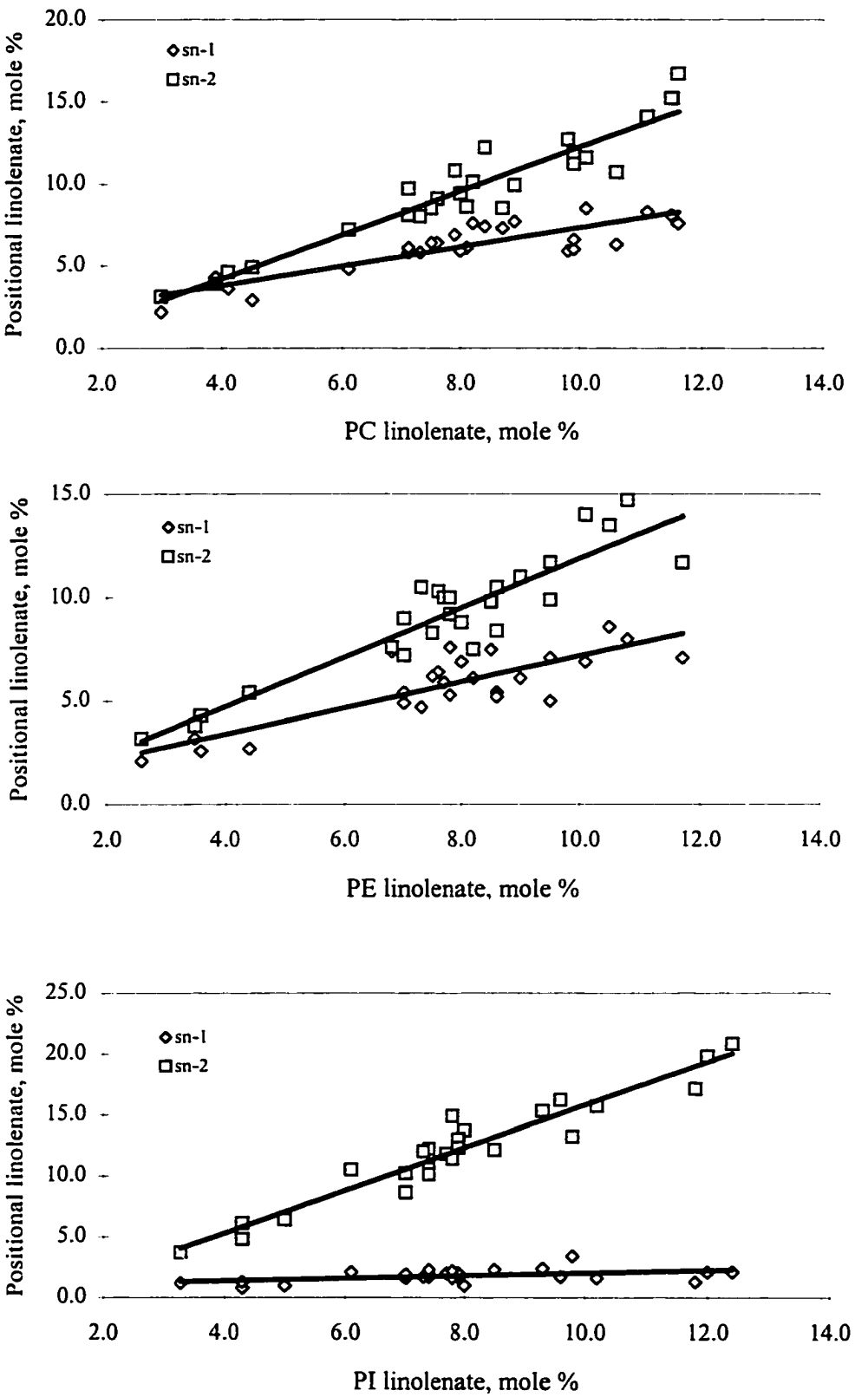


Figure 4C. PL linolenate stereospecific distribution

CHAPTER 3. SEED PHYSIOLOGICAL PERFORMANCE OF SOYBEANS WITH ALTERED SATURATED FATTY ACID CONTENTS

A paper to be submitted to *Crop Science*

Tong Wang[1], Teresa Harper[1], Earl G. Hammond[1,2], Joseph S. Burris[3], and Walter R. Fehr[4]

Abstract

Soybean seeds with elevated and reduced percentages of palmitate and elevated percentages of stearate were compared with seeds of typical-composition in tests for germination, seedling growth rate (SGR), and leachate conductivity. In general, the seed with altered compositions did well in these physiological tests, but their vigor tended to be negatively correlated with the percentage of stearate, palmitate, or total saturate levels in various lipid classes.

Key words: germination, membrane permeability, neutral lipid, phospholipids, saturated fatty acids, seedling growth rate, soybean composition modification

Introduction

Modification of the fatty acid composition of soybean oil to make it more competitive in various segments of the food and industrial oil markets (Hammond, 1992) has been an important objective of plant breeding and molecular genetics in recent years. Altered fatty acid compositions have been developed through traditional plant breeding (Wilson et al,

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1981) and application of chemical mutagens (Hammond and Fehr, 1975; Wilcox et al, 1984; Fehr et al, 1991) that have extended the range of the five major fatty acids normally found in soybean oil. Soybean oil with elevated saturated fatty acid percentages may be used to make shortening and margarine without hydrogenation, thereby reducing processing cost and avoiding the formation of *trans* fatty acids.

Aside from the triglycerides (TGs) that typically make up more than 99% of refined soybean oil, soybean seeds contain 0.3 to 0.6% phospholipids (PLs), with phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) being the major classes (Hui, 1996; Wang et al., 1997). These PLs are major components of the cell membranes that form the outer boundary of the cell and divide it into various compartments. It is important that membrane PLs have the correct balance of saturated and unsaturated fatty acids (Chapman, 1973) to be in the proper physical state for cells to perform their metabolic tasks. When oil saturate percentages were elevated, saturate levels in PLs increased (Mounts et al., 1996; Wang et al., 1997). The effect of the compositional changes in neutral lipid and PLs on soybean seed performances has not been reported, although seeds with elevated stearate frequently have abnormal field germination and reduced yield (personal communication with W.R. Fehr, Agronomy Department, Iowa State University).

In this study, we report the effect of altered saturate content in soybean seed lipids on germination, seedling growth rate, and seed electrolyte retention.

Materials and Methods

Soybean sample selection for physiological tests. Commercial soybean cultivars and experimental soybean lines were provided by W.R. Fehr at Iowa State University and Pioneer Hi-Bred International Inc. of Des Moines, IA. One hundred single seeds from each soybean line with altered palmitate or stearate content were analyzed by gas chromatography (GC) for fatty acid composition (Hammond, 1991), and twelve soybean lines were selected for the widest possible ranges of palmitate and stearate percentages and for the least variation in seed-to-seed composition.

Lipid compositional analysis. PL isolation, class separation and fatty acid composition analysis were performed as described by Wang et al. (1997).

Germination evaluation. The rolled-towel germination method (AOSA, 1986) was used. Each roll consisted of two standard weight paper towels, 35.5 x 63 cm, one below the seed and one covering the seed. Towels were moistened with 30 g water per towel. Fifty seed were oriented into two rows, with 25 seeds per row, the seeds were covered, the two paper towel were loosely rolled, and the rolls were placed in a plastic container that had partitions to hold 12 rolled towels. The containers were loosely covered with plastic bags and placed in unlighted germinators at 15°C, 25°C and 35°C for seven days, at which time germination counts were made. Three replications of all lines and temperatures were performed.

Seedling growth rate (SGR). The SGR vigor test , which evaluates the growth of seedlings from a standard germination test, was performed according to the procedures described in the Rules for Testing Seeds (AOSA, 1986). After germination count, the normal seedlings were freed of cotyledons, dried at 84°C for 63 hr, and weighed. SGR was expressed as mg/seedling.

Conductivity test. A G-2000 seed analyzer (Wavefront Inc., Ann Arbor, MI) was used to measure the electrical conductivities of the leachates from 50 single seeds. Each seed was placed in an individual cell filled with 3.5 ml distilled water on a 100-cell tray. Conductivity readings in micro-Siemens were taken after leaching for 1, 3, 6, 12, and 24 hrs. Three 50-seed replicates for each line were tested.

Results and Discussion

Fatty acids composition. Table 1 shows the average fatty acid compositions of the soybean lines tested and the standard deviations among 100 individual seeds. Two commercial cultivars, Sturdy and Kenwood, were included in the study. For the soybean lines with altered composition, there was considerably greater compositional variation among individual seeds, especially for the elevated stearate lines, than for lines with typical composition.

Seed physiological behaviors. Table 2 shows the germinations, SGRs and electrical conductivities of the leachates of 12 soybean lines. Germination percentages at 15 and 25°C were generally high except for PAL79, which had reduced saturate content and shriveled seed. Severe seed decay occurred at 35°C, making evaluation of germination at this temperature less accurate. SGR was much greater at 25 and 35°C than at 15°C, and SGR was slightly better at 25 than 35°C in most instances.

The electrical conductivity of the 24-h leachate was generally lower for typical-composition lines (300 to 330 μSiemens) than for those with altered compositions (360-600 μSiemens), except for STE07, which had a relatively high conductivity, and STE39, which had a relatively low conductivity compared to the lines with similar fatty acid compositions. PAL79 had unusually high conductivity (594 μSiemens). The conductivity was also

measured at 1, 3, 6 and 12 hr and selected results are presented in Figure 1. The conductivities at 1 and 3 hr were low (below 100 μSiemens) and similar for all lines. At 6 and 12 hr the relative conductivity difference among lines were similar to those at 24 hr. Seed lost electrolyte very rapidly from 0 to 6 hr, and the rate slowed somewhat thereafter except for PAL79, which continued to lose electrolyte.

Table 3 shows the fatty acid compositions for the experimental lines except for the control 'Sturdy'. In lines with lipids elevated in saturates there were corresponding increases in the PLs. PC and PE had higher palmitate percentages than TG except for two of the lines with elevated palmitate (PAL53 and PAL82); PE generally had more palmitate but less stearate than PC and TG except in the reduced palmitate lines; PI had the highest palmitate and stearate of the PL. PI's palmitate was higher than that of TG in all instances but PI's stearate was frequently less than those of TGs in elevated saturate lines. When one of the saturates was elevated or reduced in PLs, the other saturate tended to change in the opposite direction. These observations suggest that the saturate content of soybean PLs are regulated in some way.

Table 4 presents the linear correlations between neutral and PL saturates and germination, SGR, and conductivity. The reduced saturate line, PAL79, was not included in the analysis because of its shriveled seed and poor germination. Generally, germination percentages were negatively correlated with saturated fatty acid contents for all classes of soybean lipids. Elevated saturate lines had significantly lower germination at 25°C, especially for elevated stearate lines, which showed significant negative correlation for all lipid classes. At 35°C the stearate levels of PC and PE were significantly negatively correlated with germination.

SGRs were negatively correlated with saturate contents at 15 and 25°C for all lipid classes, although only a few were statistically significant. At 15°C, palmitate in TG and PC was more strongly negatively correlated with SGR than stearate, whereas at 25°C, stearate in TG and PI was more negatively correlated with SGR than palmitate. At 35°C, SGRs were positively correlated with palmitate and total saturate, but still negatively correlated with stearate for all lipid classes, though none of the relations at 35°C were significant. These results suggest that high palmitate may depress SGR at 15°C, and high stearate may depress SGR at both 25°C and 35°C. It was expected that soybeans with high saturate percentages should perform better at a relatively high temperature. Therefore, increased stearate percentage may be more detrimental to the viability and vigor of soybean seed than increased palmitate.

Conductivity of seed leachate was generally positively correlated with saturate content but only PC saturate was statistically significant. This indicates that with increased saturate in PC, the membrane became more permeable.

Thompson and Li (1997) reported on the effect of stearate content on seed germination of canola. When canolas were genetically modified to produce oils with greater than 30% stearate, their germination rates were lower, and seedlings were less vigorous. The suggested cause was the potential accumulation of stearate in membrane lipids, making membranes less able to adapt to changes in temperature and moisture content.

The standard germination test is the most widely accepted test for estimating seed viability, but it is an inadequate predictor of field emergence (Dornbos, 1988). Tests of seedling vigor were developed to complement viability tests. The SGR gives a reproducible and objective evaluation of differences in growth rate. The conductivity test is a measurement of electrolyte leakage from plant tissues, and the leachate conductivity has

been negatively correlated with seed viability and vigor (McKersie and Senaratna, 1983). In general, the lines with altered fatty acid compositions did well in these tests, but under some conditions, there was a significant negative correlation between elevated lipid saturates and germination, SGR and electrolyte retention. This may be related to the changes elevated saturates had caused in the physical properties of the TG and PL classes (Wang et al., 1998).

Acknowledgment

This work was supported by a research grant from Pioneer Hi-Bred International, Inc., Des Moines, IA.

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Table 1. Average soybean fatty acid percentage and composition variation (SD) among 100 seeds

Classification	Sample I.D.	16:0	SD	18:0	SD	18:1	SD	18:2	SD	18:3	SD	16:0+18:0	SD
Typical	Sturdy	10.1	0.3	4.3	1.5	24.3	2.2	52.9	1.8	8.4	0.7	14.4	1.5
	STE07	10.4	0.6	4.3	0.4	31.9	4.5	46.8	3.6	6.6	0.6	14.7	0.7
	STE15	11.1	0.5	4.0	0.3	28.3	3.8	49.3	2.7	7.3	1.1	15.1	0.5
	Kenwood	11.6	0.5	4.4	0.3	22.6	2.7	53.3	2.2	8.1	0.7	16.0	0.5
Reduced 16:0	PAL79	3.0	0.9	2.9	0.3	16.1	2.5	65.1	2.0	12.9	1.9	5.9	1.1
	PAL22	3.3	0.5	4.1	0.5	26.2	4.0	56.8	3.6	9.7	1.3	7.4	0.8
Elevated 18:0	STE39	10.1	0.5	15.6	2.6	16.7	2.0	48.3	1.9	9.4	0.9	25.6	2.3
	STE62	9.4	0.6	21.8	4.0	17.5	1.7	43.4	3.7	8.0	0.8	31.2	3.6
	STE73	8.7	0.8	25.1	5.9	17.5	1.9	40.6	4.7	8.2	0.7	33.8	5.2
	STE71	9.6	0.5	26.6	3.6	16.9	1.0	39.2	3.5	7.8	0.7	36.2	3.2
Elevated 16:0	PAL53	26.5	0.9	4.8	0.6	15.3	1.2	44.5	1.0	8.9	0.8	31.3	0.8
	PAL82	32.5	1.9	5.3	0.8	12.9	1.6	37.3	1.5	12.1	1.2	37.8	1.9

Table 2. Soybean seed germination, seedling growth rate (SGR), and conductivity tests

Sample Classification	Sample I.D.	Germination, %			SGR, mg/seedling			Conductivity, uSiemens, 24h
		15°C	25°C	35°C	15°C	25°C	35°C	
Typical	Sturdy	99.3	98.0	80.0	21.0	48.7	40.7	314.5
	STE07	96.7	95.3	76.0	22.3	48.1	43.4	431.7
	STE15	97.3	97.3	59.3	23.7	47.6	44.5	328.8
	Kenwood	98.7	96.7	46.7	23.4	50.1	43.9	305.6
Reduced 16:0	PAL79	82.7	42.0	17.3	14.3	41.4	37.9	594.4
	PAL22	98.7	94.7	72.7	22.7	43.1	40.3	364.6
Elevated 18:0	STE39	96.0	93.3	64.0	24.4	46.5	37.2	329.2
	STE62	97.3	92.7	33.3	23.2	46.3	43.1	467.7
	STE73	98.7	86.7	6.0	20.9	45.9	38.1	504.8
	STE71	88.7	88.7	38.0	17.8	40.5	42.0	363.5
Elevated 16:0	PAL53	99.3	91.3	75.3	21.9	42.1	41.6	434.8
	PAL82	95.3	82.7	26.0	19.4	47.5	52.9	519.7
	MSE	4.8	23.8	133.0	1.8	6.2	22.3	505.1
	$LSD_{0.05}$	3.7	8.2	19.4	2.2	4.2	8.0	37.8

Table 3. Neutral lipid (TG) and polar lipids (PLs) saturated fatty acid percentages for the soybean lines studied

Sample Classification	Sample I.D.	TG			PC			PE			PI		
		16:0	18:0	16:0+18:0	16:0	18:0	16:0+18:0	16:0	18:0	16:0+18:0	16:0	18:0	16:0+18:0
Typical	STE07	10.4	4.3	14.7	14.4	3.7	18.1	20.5	2.9	23.4	29.9	7.5	37.4
	STE15	11.1	4.0	15.1	15.0	3.7	18.7	20.1	2.8	22.9	32.3	6.9	39.2
	Kenwood	11.6	4.4	16.0	15.1	4.5	19.6	22.1	3.4	25.5	33.0	9.1	42.1
Reduced 16:0	PAL79	3.0	2.9	5.9	8.6	6.8	15.4	11.9	7.3	19.2	17.3	15.9	33.2
	PAL22	3.3	4.1	7.4	9.2	5.6	14.8	14.9	6.5	21.4	23.2	14.1	37.3
Elevated 18:0	STE39	10.1	15.6	25.6	11.7	8.6	20.3	18.2	5.9	24.1	24.8	15.2	40.0
	STE62	9.4	21.8	31.2	11.5	11.2	22.7	15.2	7.6	22.8	21.6	20.6	42.2
	STE73	8.7	25.1	33.8	11.2	11.9	23.1	16.0	8.3	24.3	22.2	19.3	41.5
	STE71	9.6	26.6	36.2	12.3	11.6	23.9	17.5	8.3	25.8	21.1	22.0	43.1
Elevated 16:0	PAL53	26.5	4.8	31.3	21.4	2.8	24.2	26.6	1.6	28.2	40.2	4.6	44.8
	PAL82	32.5	5.3	37.8	25.4	3.4	28.8	26.4	1.8	28.2	36.7	6.8	43.5

Table 4. Correlation (R^2) of neutral and polar lipid saturated acid percentages with the result of germination, SGR and conductivity tests[a]

	Germination			SGR			Conductivity
	15°C	25°C	35°C	15°C	25°C	35°C	
TG 16:0	-0.38	***-0.85***	-0.53	***-0.80***	-0.10	0.69	0.76
TG 18:0	-0.46	***-0.92***	-0.73	-0.62	***-0.79***	-0.55	0.52
TG 16:0+18:0	-0.43	***-0.84***	***-0.65***	***-0.63***	-0.31	0.19	0.60
PC 16:0	-0.45	***-0.82***	-0.58	***-0.81***	-0.02	0.74	0.73
PC 18:0	-0.37	***-0.90***	***-0.76***	-0.53	-0.71	-0.57	0.53
PC 16:0+18:0	-0.33	***-0.85***	-0.60	-0.63	-0.13	0.50	***0.63***
PE 16:0	0.24	-0.64	-0.44	-0.59	-0.06	0.56	0.58
PE 18:0	-0.41	***-0.91***	***-0.76***	-0.58	-0.74	-0.56	0.52
PE 16:0+18:0	-0.19	-0.60	-0.20	-0.52	-0.13	0.46	0.32
PI 16:0	-0.09	-0.48	-0.33	-0.42	-0.01	0.42	0.42
PI 18:0	-0.48	***-0.85***	-0.70	-0.57	***-0.76***	-0.48	0.47
PI 16:0+18:0	-0.20	-0.58	-0.45	-0.47	-0.26	0.28	0.34

[a] Numbers (correlation coefficients) in bold and italics are significant at 5% level.

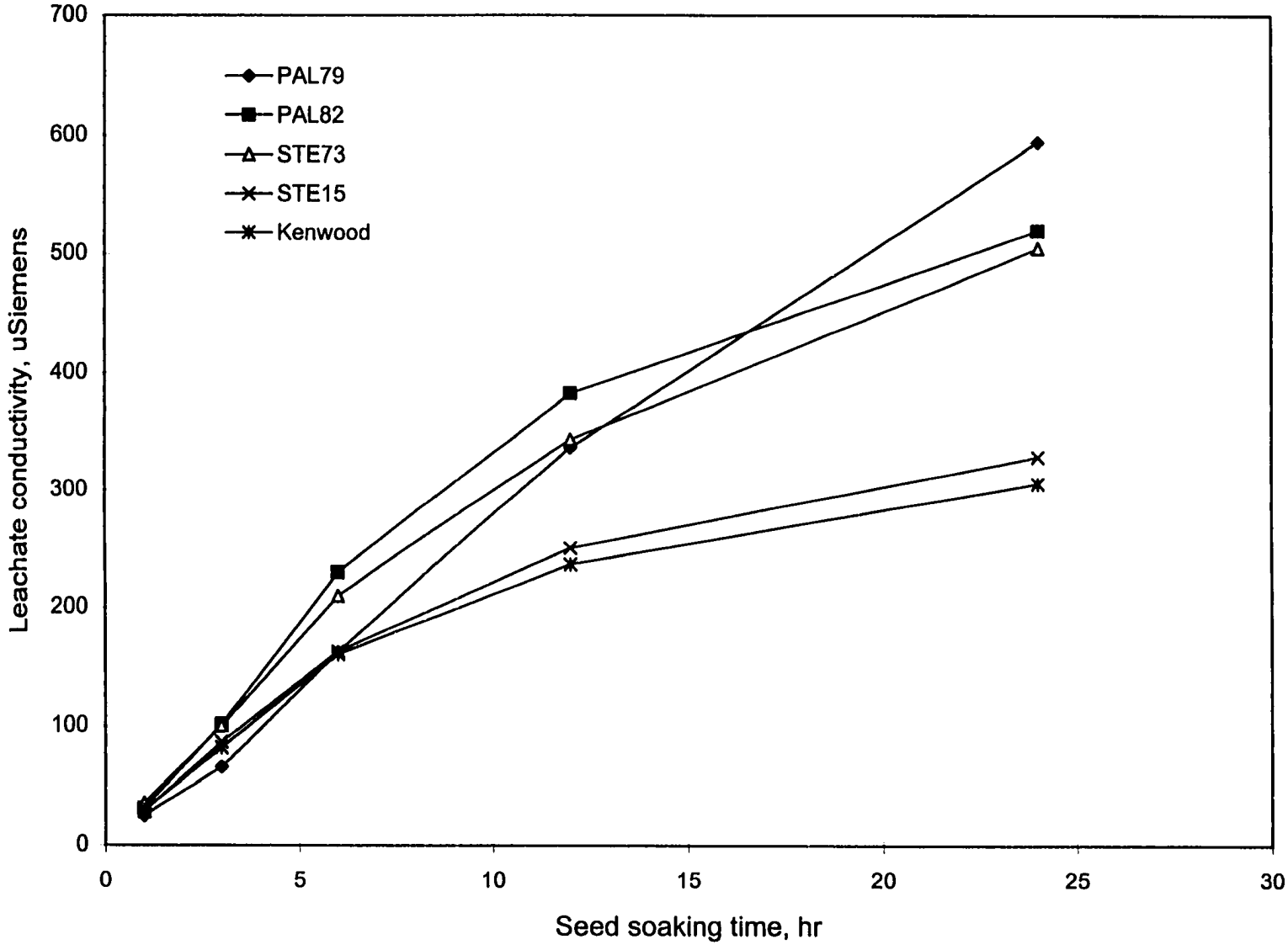

Figure 1. Electrical conductivity of leachate of selected soybean lines at various soaking time, at 23°C.

CHAPTER 4. NEUTRAL AND POLAR LIPID PHASE TRANSITION OF SOYBEANS WITH ALTERED SATURATED FATTY ACID CONTENTS

A paper to be submitted *Crop Science*

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Abstract

Increases in neutral lipid saturation may make lipids relatively unavailable as an energy source during seed germination at temperatures below its melting point. Increases in PL phase transition temperature may cause cell membrane defects that affect permeability and enzymatic activities. Soybean neutral lipid (TG) and phospholipids (PLs) were isolated, and their phase transition temperatures were measured by differential scanning calorimetry (DSC). TGs of lines with elevated stearate, elevated stearate and palmitate, elevated palmitate, typical composition, and reduced saturate had significant differences in melting temperatures. Phosphatidylcholines (PCs), the major PL in soybean, of the elevated stearate lines and lines with both saturates elevated had significantly higher phase transition temperatures than the other samples.

Key words: membrane phase transition, neutral lipid, phospholipids, saturated fatty acids, soybean composition modification

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Introduction

Soybean fatty acid composition has been modified for various applications (Hammond, 1992) through traditional plant breeding and modern molecular genetics. Soybean oil with reduced saturates may have health advantages. Oil with elevated saturates may be used for shortening and margarine manufacture without hydrogenation, thereby reducing processing cost and avoiding the formation of *trans* fatty acid. Soybeans with elevated stearate percentages have unpredictable and abnormal field germination and low yield (personal communication with W.R. Fehr, Agronomy Department, Iowa State University). Wang et al. (1998) showed that seed with altered saturated fatty acids generally did fairly well in viability and vigor tests conducted at various temperatures, but in a number of instances seed vigor was negatively correlated with saturate content, especially stearate content. Elevation of saturated fatty acids in soybean neutral lipid changes the melting point and other physical properties of the lipid, and this may cause physiological consequences.

Soybean seeds contain polar phospholipids (PLs), with phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) being the major classes (Hui, 1996, and Wang et al., 1997). These PLs are the major components of cell membranes, and it is important that these membrane PLs be in the proper physical state for cells to perform their metabolic tasks. The proper physical state requires that the PLs have the correct balance of saturated and unsaturated fatty acids (Chapman, 1973). Previous reports (Mounts et al., 1996; Wang et al., 1997) have shown when oil saturates were elevated, their levels in PLs also increased. The effects of PL composition on membrane physical properties have been studied extensively in microorganisms and animals (Ashe and Steim, 1971; Friedman and Glick, 1980; Santaren et al., 1981; Lynch and Thompson,

Jr., 1984a; Fodor et al., 1995), but there have been fewer studies on the effect of fatty acid modification on the phase transition of the major classes of plant phospholipids (Singh et al., 1977; Chen and Burris, 1991). In this paper we report the effect of saturate content of soybean seed lipids on the phase transitions of the neutral and PLs by differential scanning calorimetry (DSC).

Materials and Methods

Soybean sample selection. Eighteen soybean cultivars and experimental lines were provided by W.R. Fehr at Iowa State University and Pioneer Hi-Bred International, Inc., Des Moines, IA.

PL sample preparation. PLs isolation, class separation and fatty acid composition analysis were performed as described by Wang et al. (1997).

Membrane lipid and neutral lipid thermal phase transition by DSC. PL thermal phase transitions were measured according to Singh et al. (1977). Pure PC, PE and PI (1-5 mg) in chloroform were transferred to aluminum DSC pans, solvent was evaporated, and remaining traces of solvent was removed by placing the pans in a vacuum desiccator for 2 hr. About 8 mg of 50% ethylene glycol in water was added to the pan to hydrate the PL, and the pan was sealed and equilibrated at ambient temperature for 4 hr. Neutral lipid (TG) was extracted from crushed seed, and about 6 mg was transferred to the DSC pan for melting transition determinations. The PL samples were scanned at a 6°C/min from -70 to 30°C in a Perkin-Elmer DSC 7 instrument (Perkin-Elmer Corp., Norwalk, CT). The scan rate for neutral lipid was 5°C/min from -60 to 30°C. The melting temperature of cyclohexane was used for standardization.

Results and Discussion

Fatty acids composition. The soybean lines were classified into five groups based on their types and amounts of saturates. Table 1 shows neutral and polar lipid average fatty acid compositions of these classes. For soybean lines with typical compositions, the order of percentage of saturates was TG < PC < PE < PI. When one saturated acyl percentage (palmitate or stearate) increased in TG, its percentage in PLs also increased, although to a smaller extent, and the other saturate percentage was relatively less. When palmitate percentages were reduced in TG, it also was reduced in the PLs, but to a lesser extent than in TG. In all lines with atypical compositions, PL stearate was relatively increased. These observations suggest that saturate levels in PLs are regulated.

Neutral lipid phase transition. The phase transition temperatures of the neutral and polar soybean lipids are summarized in Table 2. The onset, peak, and terminal temperatures and melting range of the transition peak are presented in the table. The broad and shallow nature of the peaks made estimation of the peak extremes approximate. An example of one melting transition from each composition category is shown in Figure 1. TGs of lines with elevated stearate and with both elevated palmitate and stearate had melting transitions at significant higher temperatures than lines with elevated palmitate. The melting transitions of lines with elevated palmitate were at significantly higher temperatures than those with typical compositions. Lines with reduced palmitate had even lower TG melting transitions but these were not significantly different from those of typical lines.

Most TGs are polymorphic and can exist at least in three crystalline forms, designated α, β', and β. When TG samples were cooled rapidly in the DSC pans, they may have solidified in the lower melting α or β' forms. During the gradual increase in temperature, the less stable forms may have melted first and recrystallized in higher melting

forms to give the melting profiles illustrated in Figure 1. Such transitions would account for the endotherm observed in the reduced saturate sample (Figure 1e). The melting transitions in Figure 1 resembled the profile described by Roos (1995).

Neutral lipid serves as seed energy storage and should be biologically available during seed germination and seedling growth. If field germination temperature is lower than the melting temperature of TG, TG will be in a plastic, partly crystalline state and may be less accessible for metabolism, thus limiting the energy for seed germination and growth. The lines with elevated stearate would be more subject to this danger than lines with elevated palmitate because of their higher melting points. Physiological test results (Wang, et al., 1998) showed that germination and seedling growth rate (SGR) at 15 and 25°C were negatively correlated with palmitate and stearate in various lipid classes and a number of these were statistically significant. At 35°C, SGR became positively correlated with palmitate, but it was still significantly negatively correlated with stearate percentage. Therefore, elevation in stearate content seemed more detrimental to seed vigor than elevation in palmitate, and the difference in their neutral lipid melting points may partially explain this observation.

Thompson and Li (1997) reported the effect of stearate content on seed germination of canola. When canola seeds were genetically modified to produce oils with greater than 30% stearate, their germination rates were lower, and seedlings were less vigorous. One of the suggested causes was that the more saturated molecular species of canola triglycerides may be less preferred substrates for rapeseed lipase, or that the triglyceride may have crystallized into a physical state less accessible to lipases. The other possible cause was the potential accumulation of stearate in membrane lipids, making membranes less able to adapt to changes in temperature and moisture content.

Polar lipid phase transition. A series of DSC PL phase transition profiles is shown in Figure 2. The peaks were small and very broad, which is typical for biological samples (Cullis and Hope, 1991). The shapes of the transition peaks were similar for all PL samples. Generally, the low temperature inflection tended to be quite gradual, which made the determination of the onset temperature difficult, but the high temperature terminals were more abrupt. For the PL mixtures obtained from the soybean lines, there were significant differences in the temperatures of melting onset for the fatty acid composition classes but not for the peak or terminal melting point. For lines with elevated stearate percentages and those with typical compositions, melting onset occurred at higher temperatures. When the PL was fractionated, PC, which is the major fraction, showed significant differences in peak and terminal melting points with composition class. The lines with elevated stearate percentages or elevated in both stearate and palmitate percentages melted at higher temperatures. The PE showed significant differences with composition class only for the terminal melting point where lines with both elevated palmitate and stearate percentages melted higher. The PI fractions had showed significantly lower onset and peak temperatures of lines with reduced palmitate percentages than the other composition classes. Overall, the transition range of PL mixture was much wider than the individual PL classes.

Melting enthalpy was estimated to be 2 to 12 J/g PL with an average of 6 J/g, which was comparable to previously reported values (Santaren et al., 1981).

PC, PE and PI isolated from the same soybean sample had increasingly higher transition temperature as shown in Figure 2, although the average transition temperatures of PEs and PIs were not very different (Table 2). This trend in melting transitions corresponds to the percentage of saturated fatty acid in these lipid classes. PL phase

transition temperature depends primarily on acyl chain structure and only secondarily dependent on the structure of their polar head groups (McElhaney, 1994). In model PL system, for every two carbon increase in the chain length, the transition temperature increased 14°C, and for each double bond addition, the transition temperature decreased 70°C. In another model system (Van Dijck et al., 1976), PE with saturated acyl chains underwent phase transition at temperature about 20-30°C higher than the corresponding PC, because the smaller head group of PE allowed very close molecular packing. But the transition of unsaturated PE fell in the same region as the corresponding PC probably because *cis* double bonds no longer allowed close molecular packing.

The PL membrane transition temperatures obtained in this experiment were all below 0°C. DSC peak transition temperatures of isolated PCs rehydrated with ethylene glycol-water (1:1, v:v) were -13, -23 and 21°C for brain, liver and lung of chick embryo, respectively, with enthalpies ranging from 4.0 to 10.2 J/g (Santaren et al., 1981). Ladbrook and Chapman (1969) reported that the transition region for egg-yolk PC, which had over 70% of 16:0/18:1, was -15 to -5°C. Silvius (1983) reported that the peak transition temperatures of PCs with acyl chain combinations of 18:1cis Δ9/ 18:1cis Δ9, 16:0/ 18:1cis Δ 9 and 18:0/ 18:1cis Δ9 were -22, -5 and 13°C, respectively, but the transition temperatures for corresponding PEs were much higher (-16, 20, and 30.4°C, respectively).

Thus, membrane PLs from many organisms melt at temperatures well below those at which the organisms flourish, but the lack of bulk transition at growth temperatures does not imply that phase behavior is unimportant. Specialized microcrystalline regions may exist that regulate membrane function (Melchior and Steim, 1976) and require a variety of membrane lipids for proper physiological function (Mabrey and Sturtevant, 1978). Lateral phase separations of PL species in membranes have been noted (Van Dijck et al., 1976;

Cullis and Hope, 1991) and these may affect the distribution of membrane proteins (Bach, 1983). Melchior and Steim (1976) suggested that melting transitions that occur over a wide temperature range, such as those reported here for soybeans, imply that phase transition is more likely.

Numerous studies have shown that organisms adapt to changes in ambient temperature by altering the acyl composition of the PL in their membranes (Lynch and Steponkus, 1987; Dornbos et al., 1989; Maresca and Cossins, 1993; Fodor et al., 1995; Kitajka et al., 1996; Suutari et al., 1996) and this is believed to affect the fluidities and permeabilities of their membranes. But other investigators have failed to find a direct relation between acyl composition and PL thermal properties (Lynch and Thompson, 1984b) or have found only small shifts in melting properties from great changes in acyl composition (Fodor et al., 1995). The changes of melting transition documented here may account for the modest reduction in germination and seedling growth rates observed for soybean lines with elevated saturate content (Wang et al., 1998) and for the very poor field performance sometimes exhibited by soybean lines with elevated stearate percentages. But a better correlation of composition with physiological tests and a better understanding of the conditions responsible for the poor field performance are needed before a definite association can be made.

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Table 1. Neutral and polar lipid fatty acid composition (%) of various classes of modified soybeans

Lipid	Classification	# of lines	16:0	18:0	18:1	18:2	18:3
TG	High 18:0	5	10.1b	22.8a	17.3b	42.2c	7.7a
	High 16:0 & 18:0	2	24.6a	18.7b	8.6c	37.5c	10.7a
	High 16:0	5	28.0a	4.7c	13.8b	42.1c	11.4a
	Typical	3	11.4b	4.2c	26.1a	50.3b	7.9a
	Low 16:0	3	3.4c	2.6c	18.0b	64.8a	11.2a
	LSD .05		4.6	2.3	4.3	6.0	5.1
	MSE		7.3	1.8	6.2	12.4	8.8
PC	High 18:0	5	10.6c	13.0a	9.2bc	59.4ab	7.9b
	High 16:0 & 18:0	2	16.3b	11.3b	5.4c	55.8b	11.6a
	High 16:0	5	22.1a	3.1d	7.5bc	58.2ab	9.2ab
	Typical	3	13.8b	4.1d	10.1b	63.4a	7.7b
	Low 16:0	3	8.2c	6.1c	15.4a	63.3a	6.9b
	LSD .05		2.5	1.5	4.2	7.0	2.7
	MSE		2.1	0.8	5.9	16.6	2.5
PE	High 18:0	5	14.6c	9.0a	8.3b	59.8a	8.4ab
	High 16:0 & 18:0	2	21.6b	6.4b	4.1c	57.3a	10.7a
	High 16:0	5	26.0a	1.9c	5.9bc	57.6a	8.7ab
	Typical	3	19.8b	3.2c	9.4b	59.3a	7.7b
	Low 16:0	3	12.6c	5.8b	14.1a	61.3a	6.3b
	LSD .05		2.5	1.4	3.7	5.7	2.8
	MSE		2.2	0.7	4.7	11.3	2.7
PI	High 18:0	4	18.6c	21.7a	8.2a	44.1ab	7.5b
	High 16:0 & 18:0	2	29.2b	16.8b	2.8b	40.2b	11.1a
	High 16:0	4	38.5a	5.1c	4.3b	43.0b	9.2ab
	Typical	2	31.5b	8.3c	8.6a	45.1ab	6.6b
	Low 16:0	2	16.8c	17.3b	10.5a	49.5a	6.1b
	LSD .05		3.9	4.3	2.4	5.7	3.1
	MSE		3.8	4.5	1.5	7.9	2.3
Total PL	High 18:0	5	13.3c	13.5a	8.7b	56.5ab	8.0b
	High 16:0 & 18:0	2	20.0b	10.9b	4.6c	53.6b	11.2a
	High 16:0	5	25.9a	3.1e	6.5bc	55.4ab	9.1ab
	Typical	3	18.3b	4.7d	9.6b	58.9ab	7.6b
	Low 16:0	3	10.9d	8.0c	14.3a	60.0a	6.8b
	LSD .05		2.2	1.6	3.5	6.0	2.6
	MSE		1.6	0.9	4.2	12.4	2.4

Abbreviations: TG - triglyceride; PC - phosphatidylcholine; PE - phosphatidylethanolamine; PI - phosphatidylinositol; PL - phospholipid; LSD - least significant difference; and MSE - mean square error.

Table 2. Neutral and polar lipid phase transition temperatures (°C) of modified soybeans

Lipid	Classification	# of sample	Onset T[a]	Peak T[b]	Terminal T[c]	Range[d]
TG	High 18:0	5	-13.7a	18.3a	20.7a	34.4ab
	High 16:0 & 18:0	2	-17.1b	16.8a	18.9a	35.9ab
	High 16:0	5	-21.8c	8.4b	11.6b	33.4b
	Typical	3	-39.6d	-9.4c	-0.6c	39.0a
	Low 16:0	3	-46.1e	-13.8c	-8.1d	38.0ab
	LSD .05		3.3	4.6	4.1	5.0
	MSE		3.6	7.1	5.6	8.6
PC	High 18:0	5	-51.1a	-32.3a	-20.1a	31.0a
	High 16:0 & 18:0	2	-50.2a	-32.8a	-21.6a	28.7ab
	High 16:0	5	-51.7a	-37.8b	-24.3ab	27.4abc
	Typical	3	-51.3a	-38.9b	-27.4b	23.8c
	Low 16:0	3	-51.9a	-40.2b	-27.5b	24.4bc
	LSD .05		2.3	3.4	4.9	4.8
	MSE		1.8	4.0	8.0	7.8
PE	High 18:0	5	-29.8a	-10.7a	0.7ab	30.5a
	High 16:0 & 18:0	2	-32.6a	-10.0a	8.4a	40.9a
	High 16:0	5	-32.1a	-9.4a	-2.5b	29.6a
	Typical	3	-31.2a	-10.5a	-0.9b	30.3a
	Low 16:0	3	-31.5a	-9.9a	-2.2b	29.3a
	LSD .05		6.0	3.0	9.1	13.2
	MSE		12.1	3.1	28.3	59.1
PI	High 18:0	4	-21.0a	-9.2a	2.3a	23.3ab
	High 16:0 & 18:0	2	-18.9a	-9.2a	0.9a	19.8b
	High 16:0	4	-22.4a	-10.9a	0.5ab	22.9b
	Typical	2	-21.3a	-11.0a	-1.2ab	20.2b
	Low 16:0	2	-33.3b	-15.0b	-3.6b	29.7a
	LSD .05		6.8	2.6	4.1	6.4
	MSE		11.3	1.6	4.2	9.9
Total PL	High 18:0	5	-38.5a	-15.0a	1.5a	39.9a
	High 16:0 & 18:0	2	-43.9b	-17.6a	-5.2a	38.8a
	High 16:0	5	-38.8ab	-19.1a	-2.8a	36.0a
	Typical	3	-37.1a	-19.3a	-2.4a	34.7a
	Low 16:0	3	-41.8ab	-20.7a	-2.7a	39.1a
	LSD .05		5.3	6.2	6.7	10.2
	MSE		9.6	13.1	15.4	35.5

[a] Temperature of the start of the transition peak; [b] Temperature of the peak of the transition peak; [c] Temperature of the end of the transition peak; and [d] Temperature of the transition range.
For abbreviations, see table 1 footnote.

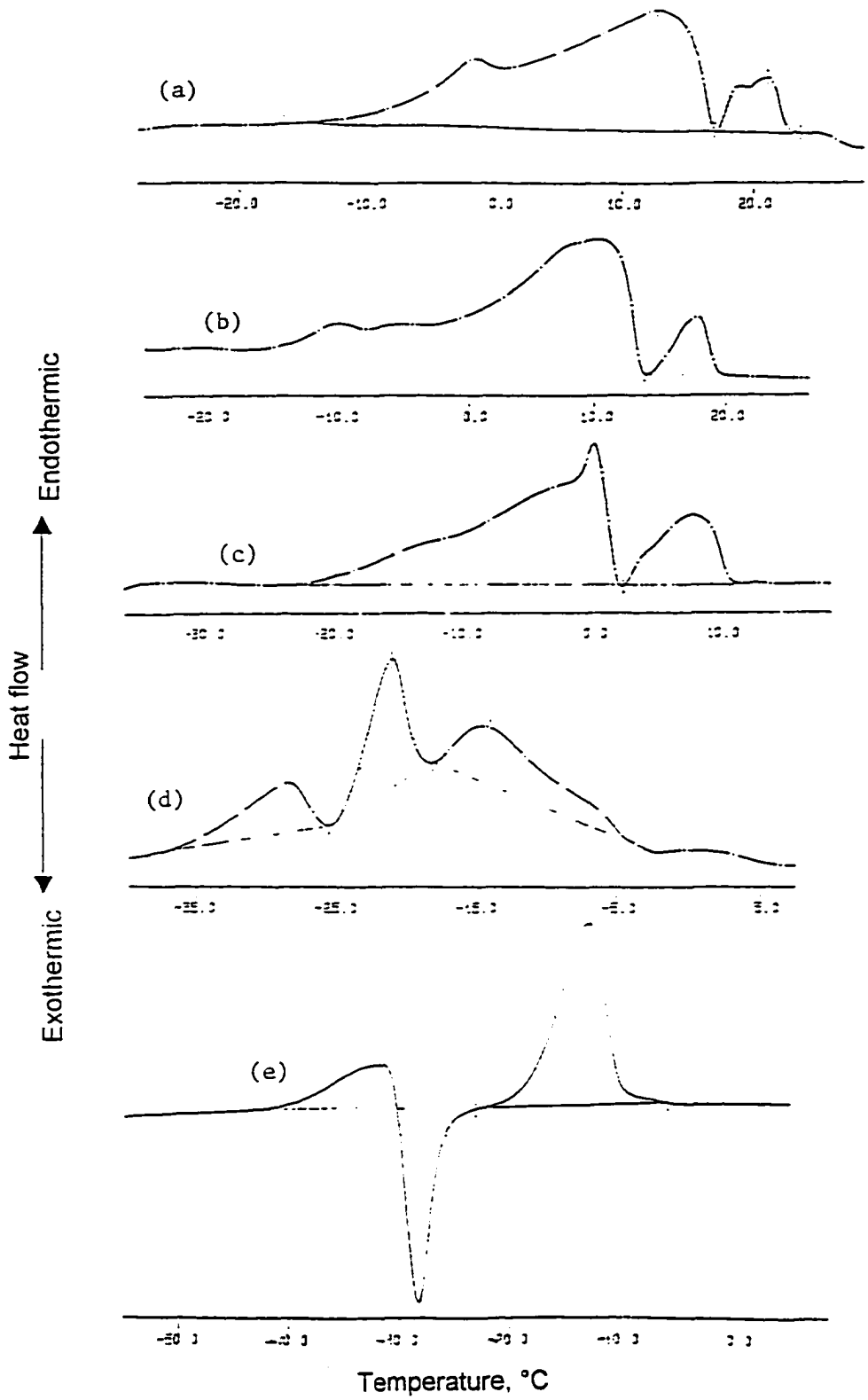

Figure 1. A schematic representation of DSC thermographs of selected soybean oil with modified fatty acid compositions. Oils were from soybeans with (a) elevated stearate, (b) elevated stearate and palmitate, (c) elevated palmitate, (d) typical composition, and (e) reduced palmitate. Scan rate was 5°C /min from -60°C to 30°C.

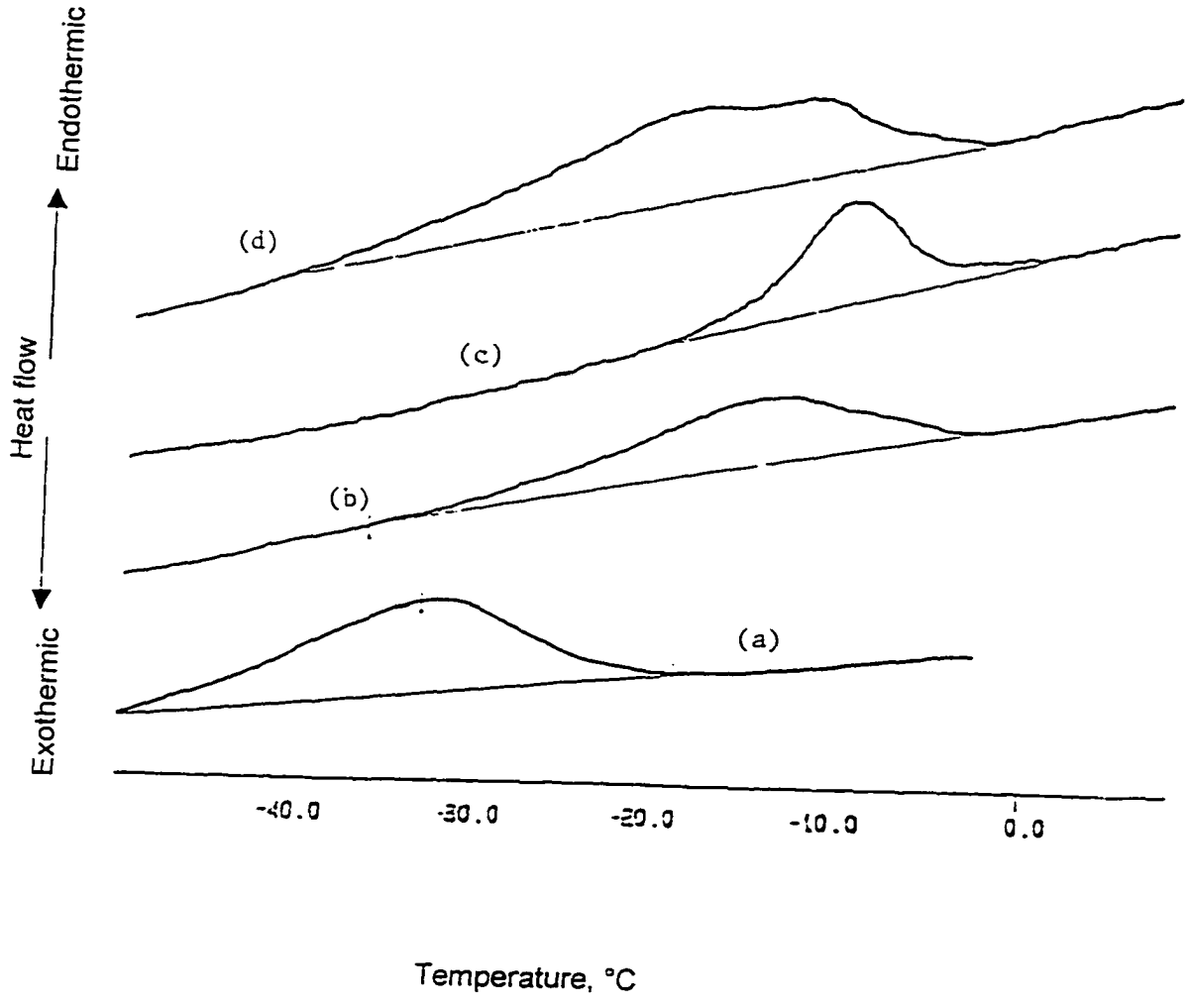

Figure 2. DSC scan of soybean phospholipids isolated from a sample with elevated palmitate percentage (33 % $C_{16:0}$). (a) phosphatidylcholine, (b) phosphatidylethanolamine, (c) phosphatidylinositol, and (d) total phospholipid. Samples were hydrated in 50% ethylene glycol in water and scanned at 6°C / min from -70°C to 30°C.

CHAPTER 5. FRACTIONATION OF PHOSPHOLIPIDS OF MODIFIED SOYBEANS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AN EVAPORATIVE LIGHT-SCATTERING DETECTOR

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Abstract

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from 23 soybean lines with a wide range of fatty acid compositions were partially resolved into molecular fractions by high-performance liquid chromatography (HPLC). A mass detector, i.e. an evaporative light-scattering detector, was used for quantification. Seven peaks were separated by HPLC, and their identities were assigned from their fatty acid compositions determined by gas chromatography (GC). The HPLC detector response was a power function of PC and PE concentrations. Various correction methods were applied to the original HPLC to obtain the best agreement between phospholipid fatty acid composition determined by GC and the composition calculated from the corrected HPLC fraction percentages. The corrected HPLC fraction composition was also compared with that was calculated from the stereospecific distribution data obtained from a previous study, using a 1-random-2-random hypothesis. Genetic modification of soybean oil composition caused changes in PL species, as shown by the correlation analysis between PL fatty acid percentages and HPLC fraction

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percentages, and this may have implications for the physical properties and physiological functions of phospholipid biomembranes.

Key words: evaporative light-scattering detection, high-performance liquid chromatography, phosphatidylcholine, phosphatidylethanolamine, phospholipids, soybean fatty acid composition modification, stereospecific distribution.

Introduction

Phospholipids (PLs) are the major component of the biological membranes, and the chemical composition of the various classes of PLs determine their physical properties, which consequently affect biological functions of the membranes (1,2). Biomembrane composition may be altered by nutritional and environmental factors (1,3-6). Genetic modification of soybean oil composition is accompanied by alteration of PL fatty acid composition (7,8), but little is known about how the molecular species composition is affected by the oil modification (7).

Three types of PL compositional information can be obtained by employing different analytical approaches. Phospholipid fatty acid composition can be determined by total hydrolysis of the ester linkages followed by gas chromatography (GC) of the fatty acid methyl ester (FAME). To obtain more structural information, stereospecific analysis can be performed by selective hydrolysis of fatty acyl group at *sn*-2 position of the individual PL class with phospholipase A2, and fatty acid composition at *sn*-1 and *sn*-2 positions can be analyzed by GC (8). To understand the combination of fatty acyl groups in PL, intact individual PL classes can be partially separated into molecular species, which provides more detailed structural data and can supplement the overall composition and

stereospecific distribution information. Soybean triglyceride molecular species have been shown to agree fairly well with that calculated by a 1-random-2-random-3-random distribution (9). It has not been determined if the PL molecular species percentages agree with that calculated from stereospecific distribution data. We had previously determined the stereospecific distribution of these modified soybeans (8), and the molecular species composition can be calculated from these data assuming a random combination of the acyl groups. This comparison can provide evidence about the 1-random-2-random distribution theory.

The most frequently used procedure for partial analysis of PL molecular species analysis has been enzymatic hydrolysis with phospholipase C to obtain 1,2-diacyl-*sn*-glycerols (DG) followed by conversion to either high-performance liquid chromatography (HPLC) or GC amenable derivatives. In HPLC applications, conversion to UV absorbing derivatives has been used frequently (10-14). In GC applications, the DGs may be converted to corresponding monoacetyldiacylglycerols (15, 16), trimethylsilyl (TMS) ethers (3, 17), or *tert*-butyldimethylsilyl (t-BDMS) ethers (18). These methods employ multi-step derivatizations that may subject the lipid to rearrangement of the fatty acyl chains on the glycerol backbone. It is also time-consuming to obtain the derivatives. Submitting underivatized /unmodified PL to chromatographic separation is the best choice for molecular species analysis. However, the general difficulty of direct HPLC molecular species analysis is associated with detection. UV detection is dependent on the number and configuration of the double bonds, and a refractive index detector is sensitive to temperature and solvent gradient change. Evaporative light-scattering detector (ELSD), a mass detector, is increasingly used in lipid analysis (7, 19-25) due to its many advantages over the other types of detector. But ELSD has a major disadvantage, which is the non-linear response to

changing quantities of the various fractions (26-29). In this paper, a method of correcting the non-linear detection is described. Although more recently, thermospray and electron spray liquid chromatography / mass spectrometry are developed to rapidly determine PL molecular species (30-32), these detectors / interfaces have their own limitations (33).

Partial analysis of molecular species is important not only for basic research, but also for food, cosmetic and pharmaceutical industries which use large amount of a PL mixture (lecithin) as an emulsifying agent. Manipulation of the molecular species composition in these lecithin preparations may provide a means of extending their range of functionalities and applications.

Experimental Procedures

Materials: Twenty three soybean lines with a wide range of fatty acid composition were obtained from Walter R. Fehr, Agronomy Department, Iowa State University and Pioneer Hi-Bred International, Inc., of Des Moines, IA. These lines were classified into six categories, and their fatty acid composition are shown in Table 1. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of these soybeans were isolated as described by Wang et al. (8) and stored at -20°C until analyzed. PC and PE standards from soybean were purchased from Sigma Chemical Co. (St. Louis, MO).

Chromatographic conditions: A HPLC system, including a Shimadzu (Columbia, MD) LC-600 solvent delivery system, a Rheodyne (Cotati, CA) 7125 injector with a 20 μL sample loop, a Phenomenex (Torrance, CA) Luna C18(2), 5μm particle size, 250 x 4.60 mm column, a Shimadzu CR501 Chromatopac integrator, and a Varex ELSD IIA (Burtonsville, Maryland), was used for PL separation and quantification. The mobile phase was methanol/chloroform/acetonitrile/water (87.5:5:3.75:3.75, by volume), which was a

modification of the solvent of Demandre et al. (13). Isocratic elution with mobile phase flow rate of 1 mL/min was performed. The Luna C18(2) column with a carbon load of 17.5% and a large surface area was selected based on the research finding of Sotirhos et al. (11). The neubulizing nitrogen gas flow rate and the temperature of the drift tube of the detector were chosen based on the recommended operating conditions from the instrument manual, which were 42 mm (equivalent to 2 L/min) and 125°C, respectively. Approximately 250 μg of PC and 150 μg of PE from all samples were injected in the system, and all analysis were performed in duplicate.

HPLC peak identification: The individual peaks were collected from the column outlet at the appropriate time while the column was disconnected from the detector. The effluent was evaporated and the lipid fraction was converted to FAME with sodium methoxide. The components of the peaks were determined by their fatty acid compositions and elution order. PL molecules with longer and more saturated acyl groups are retained on the C18 column for a longer time than those with shorter and more unsaturated acyl groups.

Standard curves and optimization of correction factors: Pure soybean PC and PE were diluted with the HPLC mobile phase to various concentrations. The concentration that gave chromatographic peaks slightly larger than the largest peaks from the sample analysis, was assigned as concentration 100, and the other concentrations were expressed as dilutions of this standard concentration. To linearize the detector response, logarithms of the peak areas were plotted against the logarithms of the normalized concentrations, and linear regression lines for each chromatographic peak of both PC and PE were obtained.

It was assumed that the peaks could be corrected by the following equation:

$$C = A^{1/x} \qquad \text{[equation 1]}$$

where C is the corrected area response, A is the observed area response, and x is the slope of the log-log linear plot. Several ways of evaluating "x" were compared: (1) x = 1; (2) x = the average slope from plots of log A versus log C for all HPLC peaks; (3) x = the individual slopes giving the best fit for each HPLC component peak; (4) x = the values of the individual HPLC peaks that gave the best agreement between the fatty acid compositions calculated from the corrected HPLC fraction percentages and the fatty acid compositions determined by GC; (5) x = the values of the individual HPLC peaks that gave the best agreement between the corrected HPLC fraction percentages and that calculated from a 1-random-2-random stereospecific distribution. A mathematical application, UNCMIN (unconditional minimization) (34), which simultaneously optimizes several parameters for a target function among all 23 sets of data using a non-linear least square principle, was used to achieve different sets of correction factors. Two target functions used were the determined fatty acid composition and the calculated molecular fraction percentages.

Calculation of molecular species composition from stereospecific distribution data: Previously determined PL stereospecific distribution data for the 23 lines was used to calculate molecular species composition assuming the 1-random-2-random distribution theory. Theoretically, there are 25 molecular species with five possible fatty acids and two possible positions for esterification. An example of such a calculation is:

% molecular species A-B = (% A at *sn*-1 x % B at *sn*-2) / 100 [equation 2]

In this situation, A-B represents the molecular species with A at the *sn*-1 position and B at the *sn*-2 position. A-B is different from B-A, in which B is at the *sn*-1 position and A is at the

sn-2 position. When A/B is used in this paper, it represents the sum of A-B and B-A.

Results and Discussion

HPLC separation and peak identification: Figure 1 shows a HPLC separation of a PC sample. A total of seven peaks were separated and identified, and they are labeled on the chromatogram. Two peaks were mixtures of two molecular fractions. They were 16:0/18:3 + 18:2/18:2, and 16:0/18:2 + 18:1/18:2, with equivalent total acyl chain lengths of 28 and 30, respectively. For a class of PL with five possible fatty acids esterified on its *sn*-1 and *sn*-2 positions, there are 25 possible molecular species. But the *sn*- positional isomers, i.e. molecular species A-B and B-A, cannot be separated by the current HPLC separation conditions. Counting A-B and B-A as one molecular fraction[1], there are 15 such fractions. The seven observed peaks included 16 out of the 25 molecular species which constitute nine molecular fractions. The other nine possible molecular species, which should make up six molecular fractions are expected to be present in very low concentrations (based on the stereospecific distribution calculation) and were not detected. In some instances, they may have been mixed with and masked by peaks of greater concentration. For instance, 18:1/18:3 could be mixed with the 16:0/18:3 + 18:2/18:2 peak, 16:0/16:0 and 18:1/18:1 might be coeluted with the peak 16:0/18:1 due to their same equivalent carbon numbers. 18:0/18:1, 16:0/18:0 and 18:0/18:0 might be eluted and detected after our last-observed

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[1] The term "fraction" will be useful for a mixture of molecular species A-B and B-A that are *sn*-1-*sn*-2 positional isomers, and for a mixture of A-B, B-A, C-D, and D-C with same equivalent carbon number. Such analyses are frequently referred to as "molecular species" analyses in the literature. In this paper, when a molecular fraction is expressed as A/B, it implies a possible mixture of A-B and B-A.

peak if they were in higher concentrations. Nevertheless, the seven observed peaks accounted for about 95% of the expected mass of the total molecular species calculated from the stereospecific distribution for both PC and PE.

Non-linear response of the light-scattering detector: When the detector response was plotted against the concentration of PC and PE standards, non-linear relationships were obtained, as illustrated in Figure 2. The plots of logarithm of peak area *vs.* the logarithm of the normalized concentration for PC and PE are presented in Figure 3. The slopes and regression coefficients (R^2) of these linear log-log relationships are summarized in Table 2. Due to the low percentage of the 18:0/18:3 fraction, this peak only appeared in the highest concentration injection and its regression can not be performed, therefore the average slope was used as the slope of this HPLC fraction.

The detector response is apparently a power function of the concentration on the basis the good log-log linear fit. This non-linear relationship can be explained by the light-scattering detection principle (26). When a peak is eluted from the column, the number of liquid droplets formed in the neubulizer per unit time is constant, whereas the concentration of the solute in these droplets varies. Thus, after solvent evaporation, the size of droplets made of the residual solute varies. When a stream of droplets passes through the detector, the amount of light scattered does not increase regularly with increasing droplet diameter, as the intensity of the scattered light increases faster than the fourth power of the diameter of the droplets (27, 28). It was observed that the detector response increased sigmoidally with increasing sample concentration (26). Thus, at low solute concentrations, the droplets scatter light to a proportionally lesser extent. As the diameter of the droplets begins to approach the wave length of the light, they no longer affect its passage and the response falls off rapidly. We actually observed relative falls on the response curves of the higher

concentration peaks when the response of a much higher concentration sample was plotted. But all our sample responses fell into the region of the curve where the response-concentration can be best illustrated by a power function.

The detector's non-linear response was also described by other researchers. Christie (26) reported a power, i.e. the slope of the linearized plot, of 1.35 for some applications, and this number was used by some manufacturer in its detector "linearizer" in an attempt to electronically improve the quantification results. Stolyhwo et al. (27) arrived at an average slope of the log-log straight lines of 1.81 ± 2% for a group of structurally different compounds, and an average slope of 1.82 ± 1.6% for a group of structurally similar compounds. Stolyhwo et al. (28) reported a slope of 1.69 for another compound. Lakritz and Jones (35) examined the detector response to a group of cholesterol oxides and obtained an average of 1.634 ± 0.024 from the log-log linearization plots. The differences in the slopes were attributed to the different designs of the detector neubulizers (26-28).

In our analysis, the slopes for various molecular fractions were different (Table 2). Possibly, the detector response is dependent on the complex refractive index, i.e. refractive index (RI) and absorption coefficient, of the lipids (26, 27). As mentioned above, Stolyhwo et al. (27) reported that the slopes for methyl esters of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$ were almost identical, and even the detector response for a group of structurally distinctively different compounds was very similar. Christie (36) reported different response curves for different classes of lipids, but the log-log linearization was not applied and the difference in response was not disscussed. The cause for different slopes of the PL molecular fractions can not be explained clearly.

Data correction for the non-linear response: The original HPLC data (areas under the peaks) need to be corrected for the non-linear response of the detector. Three

PC samples, i.e. sample #1 with typical oil composition, sample #11 with an elevated palmitate percentage, and sample #7 with an elevated stearate percentage, were used to evaluate the effectiveness of the five correction methods. The effectiveness was measured by the agreement between the fatty acid composition calculated from the corrected molecular fraction percentages and that determined by GC. The fatty acid composition was calculated as follows: If percentage of fatty acid A was to be determined, then all the peaks containing A, except for the mixture peaks, were selected and the mole percentages of the fractions were multiplied by (the number of moles of A per mole of PL) / 2, and then summed. When A was in one of the two mixture peaks, a partition between the mixed two fractions was needed. The percentage of each fraction was assumed to be equal to that calculated from the 1-random-2-random molecular species composition.

Tables 3 and 4 compare the various correction methods for PC. Table 5 presents the correction factors, i.e. the slopes of the linear regression lines or the power factor "x" in equation 1, of these methods for both PC and PE. In Table 3, the corrected molecular fraction percentages were compared with the ones calculated from the stereospecific distribution data, and the average differences (from all the samples in the same composition group) between the calculated and corrected are presented. In Table 4, average differences between the GC-determined fatty acid composition and fatty acid composition calculated from the molecular fraction percentages are presented. Fatty acid composition was also calculated from the molecular fraction percentages calculated from the 1-random-2-random hypothesis, and presented in Table 4. The uncorrected data and the data corrected with individual slopes from the standard curves had great discrepancies for both the molecular fraction composition and the fatty acid composition. When the area was corrected with the average slope, the calculated fatty acid compositions fit the GC results

fairly well, except for $C_{18:1}$ and $C_{18:3}$. The poor fit for $C_{18:1}$ may be because $C_{18:1}$ accounted for a large proportion of the 5% undetected fractions. Because of this, the $C_{18:1}$ percentages calculated from stereospecific data were also considerably different from the GC composition (Table 4). When the data was corrected with the factors (slopes) optimized for the GC-composition fit, relatively large disagreements appeared between the calculated and corrected molecular fraction composition. When the data was corrected with factors optimized for the molecular fraction composition (which was calculated based on the 1-random-2-random hypothesis), the calculated fatty acid composition fit the GC-determined data fairly well, except for $C_{18:3}$. The two optimization methods (4 and 5, i.e. for GC-composition and calculated molecular percentage fit, respectively) gave two sets of correction factors which were generally similar except for the 18:3/18:3 fraction. These two optimization methods also resulted in two sets of molecular fraction percentages which were similar in some of the major peaks (18:2/18:3 and 16:0/18:2 + 18:1/18:2), and two sets of fatty acid compositions which were similar for all the acids except $C_{18:3}$. These results suggest that the combination of the fatty acyl groups in the molecular species of PC agree fairly well with the 1-random-2-random distribution theory.

The unusually large slopes for the 18:3/18:3 fraction of both PC and PE (Table 5) were obtained when the optimization was for molecular fraction composition fit. This HPLC peak might be relatively overestimated. This peak eluted first and had a relatively narrow peak width. The later-peaks had relatively larger widths and their peak areas could be underestimated due to the detector threshold, i.e. the detection limit, which could overlook relatively larger proportions of the later-eluted peaks compared with the early-eluted peaks. Therefore, the 18:3/18:3 fraction might be highly overestimated and it needed a large correction factor to reduce its size.

Similar comparisons among the five correction methods also were made for PE, and a result similar to that of PC was obtained. The correction factors optimized for the fatty acid composition (correction 4) were therefore chosen for final PC and PE data correction.

Comparison of PL molecular fraction percentages of determined and calculated: Tables 6 and 7 compare the observed and calculated fatty acid and molecular fraction percentages for PC and PE from the 23 soybean lines. For PC, the determined 18:3/18:3 and 16:0/18:2 + 18:1/18:2 percentages were considerably greater, and the determined percentages of 16:0/18:3 + 18:2/18:2 were considerably less than the calculated ones. Similarly, for PE, the determined percentages of 16:0/18:2 + 18:1/18:2 were greater than, and the determined percentages of 16:0/18:3 + 18:2/18:2 were less than the calculated ones. These observations apply to all categories of the soybean samples, and suggest that the combination of acyl chains may not be totally random. This may be partially explained by the non-coincidence of the peak biosynthesis of different fatty acids during maturation of the beans, which was accounted for the discrepancies between the determined and the calculated triglyceride molecular fraction composition assuming 1-random-2-random-3-random distribution (9). At different seed developmental stages, the relative availability of various fatty acyl groups for PL synthesis may be different. Although the combination of acyl groups may be 1-random-2-random at particular stage, the overall distribution at maturity may be deviated from random. Different types of soybeans, for instance, soybeans with elevated stearate and with elevated or reduced palmitate, had different fatty acid composition profiles during maturation (37, 38), and this may further complicate the verification of the 1-random-2-random hypothesis.

In all PC and PE samples, 16:0/18:3 +18:2/18:2, 16:0/18:2 + 18:1/18:2, 18:2/18:3, and 18:0/18:2 were the major molecular fractions. 16:0/18:1 was present in higher

concentration in PE than in PC. Nishikara and Kito (14) reported that 16:0/18:2, 18:0/18:2, 18:1/18:2, and 18:2/18:2 were the major fractions of PC and PE in soybean hypocotyl and cotyledon.

Effect of soybean oil fatty acid composition on PL molecular species composition: When soybean oil composition is modified, the fatty acid composition of PL is changed in a similar fashion but to a smaller extent than the triglyceride (8). The overall PL fatty acid composition is a reflection of the detailed molecular species composition. Table 8 shows the correlation among various fatty acid percentages for PC and PE, and Table 9 shows how the seven HPLC fraction percentages correlate with the PL fatty acid percentage. When $C_{16:0}$ was elevated, the percentage of 16:0/18:3 + 18:2/18:2 decreased significantly, because $C_{16:0}$ and $C_{18:2}$ were negatively correlated and about 90% of this fraction was 18:2/18:2. $C_{16:0}$ and the fraction 16:0/18:2 + 18:1/18:2 showed a significant positive correlation, owing to 16:0/18:2 being about 70% of this fraction. With increased $C_{16:0}$, the fractions containing $C_{18:0}$ decreased significantly in almost all cases, reflecting the negative correlation between the acyl groups. When $C_{18:0}$ was elevated, all fractions containing $C_{18:0}$ increased, but the fraction 16:0/18:2 + 18:1/18:2 decreased significantly. These observations suggested that molecular species containing saturated fatty acids were regulated in some way to balance the total saturated species content. When $C_{18:1}$ was elevated, only 16:0/18:1 showed an increase. If $C_{18:2}$ was elevated, the fraction 16:0/18:3 + 18:2/18:2 increased significantly, but the 18:0/18:2 fraction did not change. $C_{18:2}$ and $C_{18:3}$ in PL were significantly negatively correlated and this may explain that with an increase of $C_{18:2}$, 18:2/18:3 and 18:3/18:3 fractions decreased significantly. When $C_{18:3}$ was elevated, 18:3/18:3 and 18:2/18:3 fractions increased significantly, but the fraction 16:0/18:3 + 18:2/18:2 decreased significantly, which may again be explained by the fatty acid correlation

analysis. This evidence show how genetic modification of soybean oil and consequently PL fatty acid composition caused changes in PL molecular fraction composition.

There is little information available on soybean PL molecular fraction quantification. Mounts et al. (7) seemed to be the only author who reported the effect of genetic modification on molecular fraction percentages of soybean PC and PE. But their HPLC separation profile was quite different from the current study, and the light-scattering detector response was assumed linear (39). Therefore, our results cannot be satisfactorily compared to theirs.

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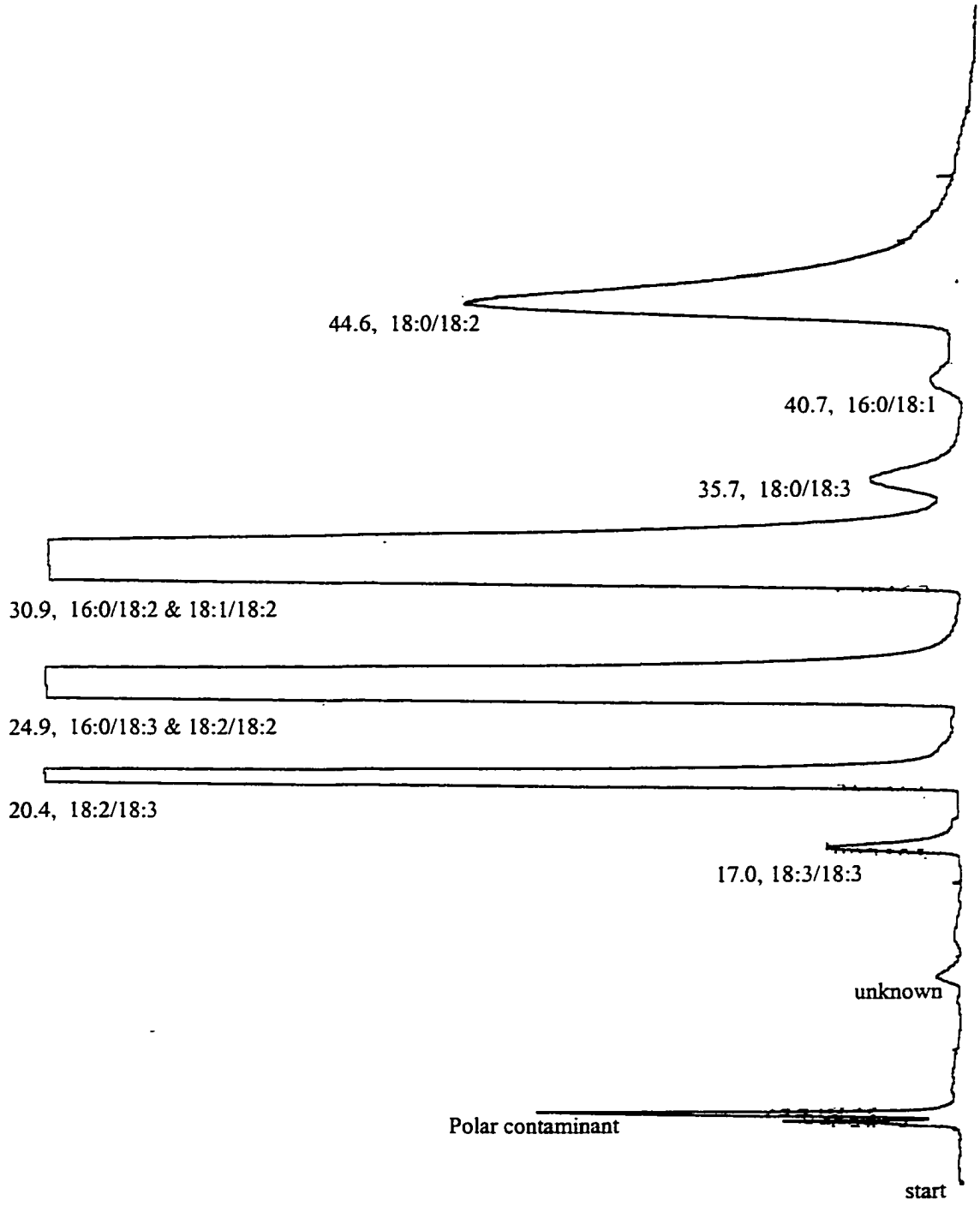

Figure 1. HPLC separation of PC of sample #5 on a Luna C18(2) column, with methanol/chloroform/acetonitrile/water (87.5:5:3.75:3.75, by volume) as mobile phase. An evaporative light-scattering detector was used for quantification. Peak retention times (min) and identities determined by fatty acid composition with GC were labeled on the chromatogram.

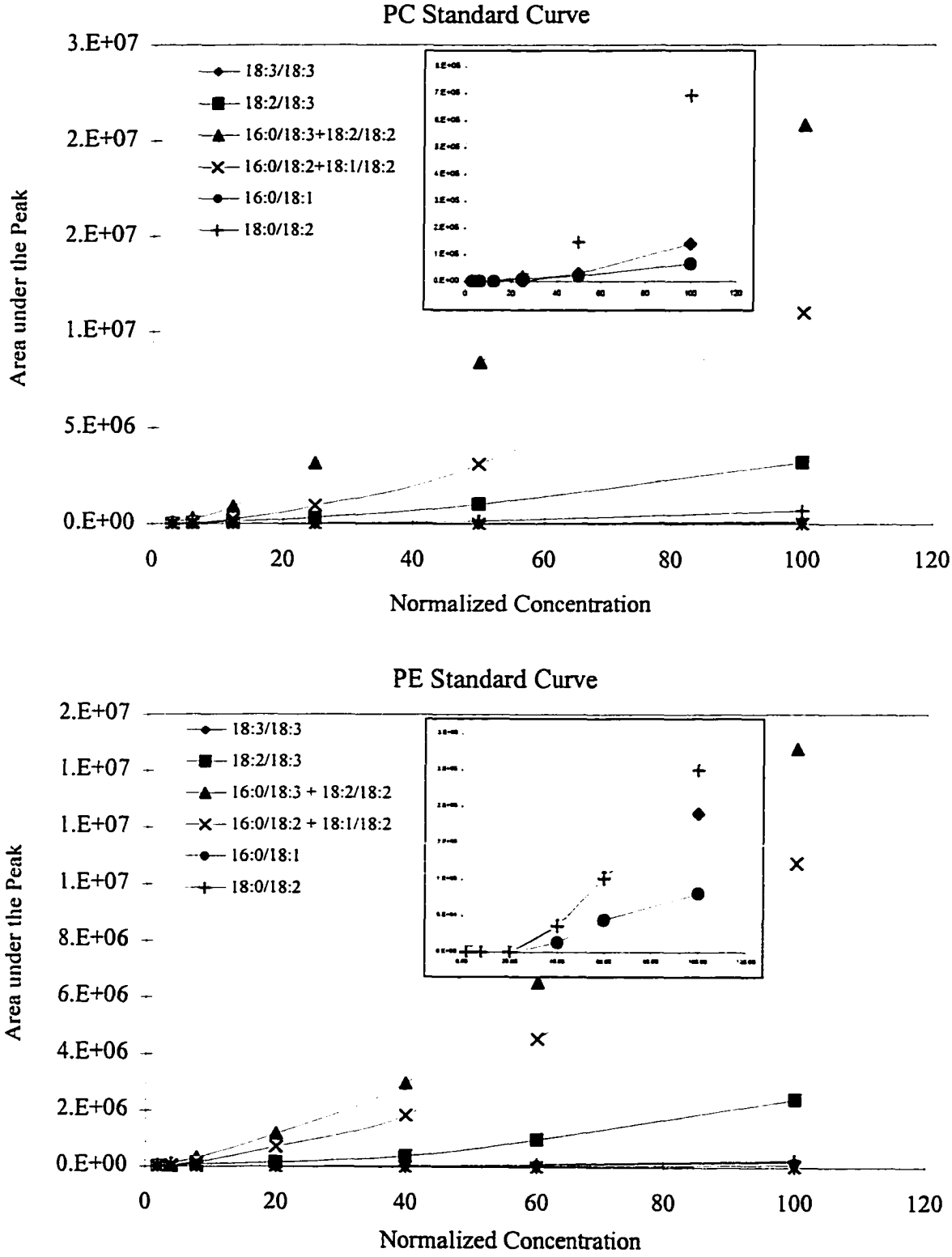

Figure 2. Light-scattering detector response of PC and PE standards. The highest concentration used was assigned a value of 100, and the other dilutions were normalized accordingly. Insets are for peaks with low concentrations.

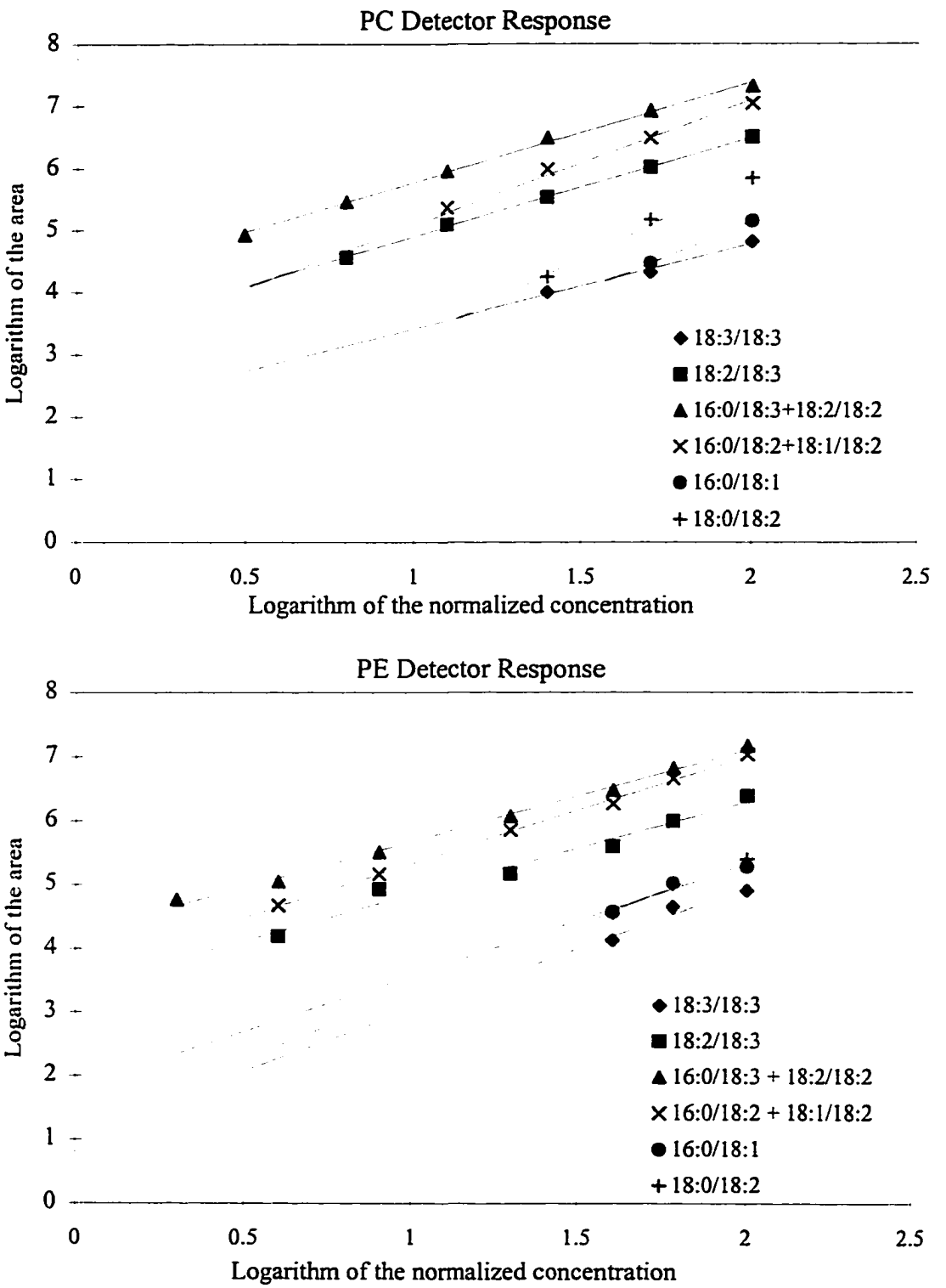

Figure 3. Log-log linearization plots of the light-scattering detector response for PC and PE standards.

Table 1. Fatty acid composition of modified soybeans, mole %

Category	I.D.	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Typical	1	12.9	4.3	22.3	52.5	8.0
	2	10.1	4.3	24.3	52.9	8.4
	3	11.4	4.1	30.8	46.5	7.1
	4	11.6	4.2	28.8	48.3	7.1
High $C_{16:0}$	11	33.3	7.3	11.7	35.3	12.4
	12	28.8	4.5	14.6	43.4	8.8
	A17	16.0	4.3	24.8	51.2	3.6
	A19	28.8	4.0	14.0	42.9	10.4
	A21	20.8	4.0	17.4	48.9	8.9
	A24	16.7	4.1	19.4	48.1	11.7
	spf358	28.4	3.9	11.3	40.1	16.3
High $C_{18:0}$	5	12.0	15.1	16.4	47.6	9.0
	6	10.6	20.3	16.8	44.0	8.3
	7	10.7	23.9	16.5	41.1	7.8
	8	11.8	21.8	17.2	41.0	8.3
	stea	8.2	23.6	18.1	42.3	7.8
High $C_{16:0}$ & $C_{18:0}$	453	23.6	17.3	9.4	38.3	11.5
	561	25.6	20.1	7.8	36.7	9.8
Low $C_{16:0}$	10	3.1	2.7	15.4	64.8	13.9
	spf457	3.4	2.8	21.0	68.3	4.5
	spe153	3.6	2.4	17.5	61.4	15.1
Low $C_{18:3}$	A5	11.0	4.2	35.3	44.5	4.9
	spb201	10.2	4.6	26.9	55.6	2.7

Table 2. Slopes and regression coefficients of the log-log linearization of the detector response for PC and PE standards

		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	16:0/18:1	18:0/18:2
PC	Slope	1.361	1.599	1.606	2.021	2.267	2.649
	R^2	0.984	0.999	0.997	0.992	1.000	0.992
PE	Slope	1.923	1.445	1.433	1.675	1.748	2.110
	R^2	0.935	0.970	0.996	0.998	0.955	0.988

Table 3. Comparison of mole percentages of HPLC peaks with those calculated by the 1-random-2-random hypothesis using various correction methods for PCs of typical oil composition (#1), elevated C16:0 (#11),and elevated C18:0 (#7) samples

Sample	Corr. method[a]		18:3/18:3	18:2/18:3	6:0/18:3 + 18:2/18:2	6:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
#1		calc.	0.6	9.5	40.0	38.7	0.7	4.1	6.3
	1		0.2	7.1	54.2	35.0	-	1.1	2.4
		avg. diff.[b]	0.4	1.9	-14.5	4.3	0.8	2.6	4.7
	2		2.0	13.7	39.9	31.7	-	5.3	7.5
		avg. diff.	-1.7	-4.8	0.9	6.3	0.4	-1.0	-0.1
	3		5.9	19.4	66.7	7.0	-	0.7	0.4
		avg. diff.	-6.5	-10.1	-23.6	30.4	0.7	3.0	6.1
	4		2.6	7.0	33.7	46.5	-	1.5	8.7
		avg. diff.	-2.5	2.4	6.7	-8.3	0.7	2.2	-1.2
	5		0.2	7.1	39.7	41.7	-	3.9	7.5
		avg. diff.	0.5	2.3	0.4	-3.8	0.7	0.1	-0.1
#11		calc.	0.8	9.3	32.9	46.7	0.9	4.8	4.8
	1		0.4	8.0	39.6	48.7	0.1	1.7	1.5
		avg. diff.	0.4	2.1	-9.5	0.2	0.6	2.5	3.6
	2		2.8	14.2	32.9	36.6	1.2	6.4	5.9
		avg. diff.	-1.7	-4.6	1.0	6.0	0.5	-0.7	-0.4
	3		11.4	21.9	57.4	7.9	0.4	0.8	0.3
		avg. diff.	-7.6	-10.4	-23.6	33.9	0.6	2.7	4.4
	4		3.9	6.8	26.7	54.1	0.2	1.6	6.7
		avg. diff.	-2.5	2.7	8.1	-10.0	0.6	2.1	-1.0
	5		0.2	7.1	32.5	49.4	0.3	4.7	5.9
		avg. diff.	0.6	2.5	1.6	-5.3	0.6	0.3	-0.3
#7		calc.	0.9	12.3	42.2	24.6	2.6	1.6	15.8
	1		0.3	9.4	57.4	21.1	1.1	0.3	10.3
		avg. diff.	0.5	1.5	-15.8	4.9	1.4	2.0	5.6
	2		2.4	14.6	37.9	22.5	4.8	2.5	15.3
		avg. diff.	-1.7	-3.6	2.1	4.8	-2.1	-0.6	1.1
	3		7.6	21.1	63.3	5.2	1.7	0.4	0.7
		avg. diff.	-7.5	-10.4	-23.7	22.9	0.9	2.0	15.8
	4		3.4	7.9	33.7	33.3	0.7	0.8	20.1
		avg. diff.	-2.7	3.1	6.5	-6.6	1.8	1.5	-3.6
	5		0.2	8.2	40.6	30.9	0.9	2.0	17.1
		avg. diff.	0.6	2.8	-0.3	-4.1	1.6	0.0	-0.7

[a] various correction methods are explained in the footnote of Table 5.

[b] average difference between the calculated and the corrected molecular fractionation percentages (mole) of 4 soybean lines with typical composition, 7 lines with elevated $C_{16:0}$, and 5 lines with elevated $C_{18:0}$.

Table 4. Comparison of fatty acid composition (mole %) between GC-determined and those calculated from the HPLC-determined molecular fraction percentages using various correction methods for PCs of typical oil composition (#1), elevated C16:0 (#11), and elevated C18:0 (#7) samples

Sample	Corr. method[a]		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
#1		GC deter.	15.1	4.5	11.5	61.3	7.6
		calc. by stereo-dist	15.9	3.5	9.1	64.2	7.3
	1		13.8	1.2	6.9	72.3	5.8
		avg. diff.[b]	1.8	3.3	4.2	-10.8	1.7
	2		14.2	3.7	8.4	63.3	10.4
		avg. diff.	1.1	0.7	2.8	-1.1	-3.4
	3		5.1	0.2	1.6	75.0	18.1
		avg. diff.	10.0	3.9	9.1	-11.4	-11.6
	4		16.8	4.4	9.2	62.2	7.4
		avg. diff.	-1.7	0.3	1.7	0.0	-0.3
	5		16.7	3.7	9.5	64.8	5.2
		avg. diff.	-1.6	0.8	1.5	-3.2	2.4
#11		GC deter.	25.4	3.4	8.1	53.4	9.8
		calc. by stereo-dist	24.5	2.8	7.0	56.4	9.3
	1		24.5	0.8	5.7	60.5	8.5
		avg. diff.	1.6	2.4	2.1	-7.7	1.7
	2		21.3	3.6	6.8	54.3	14.0
		avg. diff.	2.8	0.3	1.1	-0.9	-3.2
	3		9.5	0.4	1.2	60.5	28.5
		avg. diff.	13.9	2.8	6.0	-9.2	-13.4
	4		25.3	3.4	6.2	54.9	10.2
		avg. diff.	-1.5	0.1	0.7	0.7	0.1
	5		25.6	3.1	7.3	56.9	7.2
		avg. diff.	-1.0	0.4	0.3	-2.3	2.7
#7		GC deter.	12.3	11.6	8.5	59.3	8.4
		calc. by stereo-dist	10.0	9.2	5.2	65.9	9.7
	1		8.8	5.7	3.9	74.3	7.3
		avg. diff.	1.7	5.5	4.4	-12.8	1.4
	2		9.7	10.1	5.2	61.7	13.3
		avg. diff.	1.0	1.5	3.1	-1.0	-4.4
	3		3.8	1.2	1.1	72.9	21.0
		avg. diff.	7.2	10.3	7.7	-11.9	-13.1
	4		12.2	10.4	6.3	62.3	8.7
		avg. diff.	-1.4	1.0	2.0	-1.5	0.0
	5		12.2	9.0	6.5	66.2	6.0
		avg. diff.	-1.5	2.4	1.8	-5.3	2.8

[a] various correction methods are explained in the footnote of Table 5.

[b] average difference between the GC-determined and that calculated from HPLC fraction percentages of 4 soybean lines with typical composition, 7 lines with elevated $C_{16:0}$, and 5 lines with elevated $C_{18:0}$.

Table 5. Correction factors (slopes of the log-log linearization of the detector response) used for various ways of data correction for PC and PE

	Corr. Method	18:3/18:3	18:2/18:3	16:0/18:3 & 18:2/18:2	16:0/18:2 & 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
PC								
	1[a]	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2[b]	1.917	1.917	1.917	1.917	1.917	1.917	1.917
	3[c]	1.361	1.599	1.606	2.021	1.917	2.267	2.649
	4[d]	1.688	1.975	1.852	1.735	2.533	2.191	1.754
	5[e]	3.202	1.999	1.838	1.777	2.473	1.897	1.818
PE								
	1[a]	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2[b]	1.722	1.722	1.722	1.722	1.722	1.722	1.722
	3[c]	1.923	1.445	1.433	1.675	1.722	1.748	2.110
	4[d]	1.928	1.737	1.731	1.640	2.055	1.659	1.655
	5[e]	10.526	2.176	1.930	1.839	2.441	2.288	1.927

[a] no correction factor applied.

[b] an average slope from the standard curve used to correct original data.

[c] individual slopes from the standard curve used to correct original data.

[d] optimized factors (x in equation 1) for best GC composition fit used to correct original data.

[e] optimized factors (x in equation 1) for best HPLC fraction percentage fit used to correct original data.

Table 6. Comparison of fatty acid composition (mole %) determined by GC (D) with that calculated from HPLC molecular fraction percentages (C), and a comparison of the mole percentages of the HPLC peaks (D) with that calculated (C) by the 1-random-2-random hypothesis for PC. Factors optimized for the best GC fatty acid composition were used to correct the areas.

category	I.D.		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
Typical	1	D	15.1	4.5	11.5	61.3	7.6	C	0.6	9.5	40.0	38.7	0.7	4.1	6.3
		C	16.8	4.4	9.2	62.2	7.4	D	2.6	7.0	33.7	46.5	-	1.5	8.7
	2	D	13.8	4.8	13.8	59.8	7.8	C	0.6	9.2	38.3	39.0	0.9	4.7	7.3
		C	15.1	4.4	11.2	60.7	8.6	D	3.7	7.4	31.7	46.6	0.1	1.8	8.6
	3	D	14.4	3.7	8.8	65.8	7.5	C	0.5	9.8	44.1	35.6	0.7	2.9	6.3
		C	17.0	3.9	7.6	63.4	8.0	D	3.1	7.6	35.7	44.8	0.2	1.2	7.5
	4	D	15.0	3.7	8.3	64.2	8.9	C	0.8	11.5	44.3	34.3	0.7	2.5	5.9
		C	16.2	2.8	7.4	64.7	8.8	D	3.3	8.4	38.7	43.0	-	0.8	5.6
	avg. diff.		-1.7	0.3	1.7	0.0	-0.3		-2.5	2.4	6.7	-8.3	0.7	2.2	-1.2
	std. dev.		0.7	0.4	0.9	1.6	0.5		0.5	0.5	1.2	0.8	0.1	0.6	1.1
High $C_{16:0}$	11	D	25.4	3.4	8.1	53.4	9.8	C	0.8	9.3	32.9	46.7	0.9	4.8	4.8
		C	25.3	3.4	6.2	54.9	10.2	D	3.9	6.8	26.7	54.1	0.2	1.6	6.7
	12	D	21.4	2.8	6.9	61.0	7.9	C	0.7	11.2	44.6	37.3	0.4	2.3	3.5
		C	21.3	3.0	7.2	60.2	8.2	D	3.5	6.9	30.3	52.1	-	1.1	6.1
	A17	D	17.5	2.7	6.2	69.1	4.5	C	0.1	5.3	47.7	39.2	0.3	2.4	5.0
		C	20.1	2.9	6.3	66.9	3.7	D	0.5	4.8	38.1	50.0	-	0.7	5.8
	A19	D	21.0	2.9	6.4	60.0	9.9	C	0.9	10.3	37.5	42.3	0.9	3.1	5.0
		C	23.3	2.5	5.5	58.7	10.0	D	3.8	7.8	31.5	51.1	-	0.9	5.0
	A21	D	20.2	3.3	9.2	60.2	7.1	C	0.6	9.5	39.7	41.0	0.6	3.7	4.9
		C	20.7	2.5	7.5	60.9	8.4	D	3.3	7.2	32.4	51.0	-	1.1	5.0
	A24	D	16.5	2.3	5.5	65.3	10.6	C	0.7	10.2	40.9	39.2	0.7	2.9	5.4
		C	20.2	3.3	6.4	61.4	8.7	D	3.2	7.7	33.7	47.9	-	1.0	6.6
	spf358	D	22.4	3.2	7.1	56.4	11.1	C	1.3	12.2	36.6	41.9	0.7	3.2	4.1
		C	24.0	2.3	5.6	57.0	11.1	D	4.5	7.7	30.4	51.7	-	1.1	4.6
	avg. diff.		-1.5	0.1	0.7	0.7	0.1		-2.5	2.7	8.1	-10.0	0.6	2.1	-1.0
	std. dev.		1.5	0.7	1.1	1.9	1.0		1.0	1.4	3.0	2.4	0.2	0.6	0.9
High $C_{18:0}$	5	D	11.7	8.6	6.6	63.2	10.1	C	1.0	12.9	43.5	28.0	1.7	1.9	11.1
		C	13.9	8.3	5.9	62.0	10.0	D	4.3	8.6	33.9	36.0	0.5	0.7	16.0

Table 6. (continued)

	6	D	11.5	11.2	11.5	57.8	8.2	C	0.9	10.8	38.3	30.7	2.0	3.2	14.1
		C	11.9	9.9	8.8	61.3	8.1	D	3.1	7.4	31.4	37.0	0.6	1.2	19.2
	7	D	12.3	11.6	8.5	59.3	8.4	C	0.9	12.3	42.2	24.6	2.6	1.6	15.8
		C	12.2	10.4	6.3	62.3	8.7	D	3.4	7.9	33.7	33.3	0.7	0.8	20.1
	8	D	11.2	11.9	8.6	58.6	9.9	C	0.8	9.4	33.0	31.7	2.9	3.2	19.1
		C	14.1	10.5	5.9	59.9	9.6	D	3.8	7.6	31.9	34.6	0.8	1.1	20.2
	stea	D	9.6	13.9	9.6	59.8	7.3	C	0.6	8.4	36.1	26.9	2.8	2.5	22.7
		C	11.0	13.0	7.7	60.9	7.3	D	2.9	6.6	29.7	33.8	0.7	1.1	25.4
	avg. diff.		-1.4	1.0	2.0	-1.5	0.0		-2.7	3.1	6.5	-6.6	1.8	1.5	-3.6
	std. dev.		1.2	0.4	0.8	1.9	0.2		0.5	1.3	3.3	2.2	0.5	0.6	1.7
High $C_{16:0}$ & $C_{18:0}$	453	D	16.5	11.7	7.3	53.3	11.5	C	1.3	11.9	32.7	33.0	3.2	3.1	14.9
		C	18.3	9.8	5.2	55.5	11.2	D	5.0	7.6	26.4	40.5	0.7	1.0	18.9
	561	D	16.0	10.8	3.4	58.3	11.6	C	1.4	12.6	35.3	29.7	3.7	1.2	16.2
		C	19.1	9.7	2.9	56.2	12.1	D	5.4	8.2	28.2	38.3	0.6	0.6	18.7
	avg. diff.		-2.5	1.5	1.3	-0.1	-0.1		-3.9	4.4	6.7	-8.1	2.8	1.3	-3.2
	std. dev.		1.0	0.6	1.1	3.0	0.5		0.2	0.0	0.7	0.8	0.4	1.1	1.1
Low $C_{16:0}$	10	D	8.6	6.8	16.2	60.3	8.1	C	0.6	9.3	39.0	35.1	1.4	3.6	11.0
		C	9.3	6.5	12.9	63.6	7.7	D	3.0	7.6	34.7	40.9	0.4	0.9	12.5
	spf457	D	7.0	6.0	10.1	73.0	3.9	C	0.2	6.1	51.0	29.3	0.5	2.0	10.8
		C	8.5	5.6	10.5	70.7	4.7	D	1.8	5.2	44.4	37.5	-	-	11.1
	spe153	D	9.1	5.6	19.9	56.7	8.7	C	0.7	9.7	35.3	39.0	1.4	5.1	8.8
		C	9.7	5.5	14.8	61.8	8.2	D	3.3	7.6	32.3	44.5	0.3	1.3	10.8
	avg. diff.		-0.9	0.3	2.7	-2.0	0.0		-2.2	1.5	4.7	-6.5	0.9	2.9	-1.3
	std. dev.		0.5	0.2	2.8	3.9	0.7		0.6	0.6	1.8	1.4	0.3	0.9	0.9
Low $C_{18:3}$	A5	D	13.3	3.8	12.0	66.6	4.1	C	0.2	5.5	44.8	38.6	0.4	4.2	6.3
		C	15.0	4.3	10.7	65.8	4.2	D	1.2	4.9	36.2	47.9	-	1.2	8.6
	spb201	D	13.3	4.2	15.3	64.3	3.0	C	0.1	3.4	40.6	42.7	0.3	5.4	7.4
		C	14.1	4.4	12.5	64.4	4.7	D	2.5	3.5	34.2	49.6	-	1.4	8.8
	avg. diff.		-1.3	-0.3	2.1	0.4	-0.9		-1.7	0.2	7.5	-8.1	0.4	3.5	-1.8
	std. dev.		0.6	0.2	1.1	0.6	1.1		1.0	0.5	1.5	1.6	0.1	0.8	0.7

Table 7. Comparison of fatty acid composition (mole %) determined by GC (D) with that calculated from HPLC molecular percentages (C), and a comparison of the mole percentages of the HPLC peaks (D) with that predicted (C) by the 1-random-2-random hypothesis for PE. Factors optimized for the best GC composition were used to correct the areas.

category	I.D.		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
Typical	1	D	22.1	3.4	9.4	56.8	8.6	C	0.5	7.6	34.6	46.6	0.6	5.3	4.8
		C	24.5	2.6	8.5	58.1	6.4	D	0.4	8.4	30.2	49.3	-	6.6	5.1
	2	D	20.2	3.6	11.4	57.2	7.5	C	0.5	8.2	35.1	44.1	0.7	5.8	5.6
		C	22.4	2.9	10.7	56.7	7.3	D	0.9	9.7	28.8	46.7	-	8.1	5.8
	3	D	20.6	2.9	9.6	60.1	6.8	C	0.6	9.6	40.5	41.6	0.4	3.6	3.6
		C	22.8	2.7	7.2	60.5	6.8	D	0.8	9.2	32.7	46.9	-	5.1	5.3
	4	D	20.1	2.8	7.9	61.5	7.8	C	0.7	10.2	38.9	42.5	0.5	3.3	3.9
		C	23.7	2.8	6.5	60.0	7.0	D	0.9	8.9	32.1	48.1	-	4.4	5.6
	avg. diff.		-2.6	0.4	1.4	0.1	0.8		-0.1	-0.1	6.3	-4.0	0.6	-1.6	-1.0
	std. dev.		0.7	0.4	0.8	1.2	1.0		0.2	1.2	1.4	1.6	0.1	0.6	0.8
High $C_{16:0}$	11	D	26.4	1.8	7.2	55.2	9.5	C	0.6	8.5	32.7	46.7	1.1	4.5	5.9
		C	27.8	2.2	7.1	54.6	8.3	D	0.8	9.2	27.1	52.5	0.4	6.0	3.9
	12	D	26.6	1.6	5.0	59.6	7.3	C	0.5	8.0	37.1	48.4	0.3	2.9	2.9
		C	28.0	1.8	5.3	57.7	7.2	D	0.7	8.5	29.1	53.7	-	4.2	3.7
	A17	D	23.0	1.5	4.3	66.9	4.4	C	0.1	5.0	44.5	45.1	0.2	2.2	2.9
		C	24.5	2.0	4.9	64.7	3.9	D	0.0	5.7	36.0	51.5	-	2.7	4.0
	A19	D	26.8	2.1	5.3	56.9	9.0	C	0.8	9.1	35.2	48.1	0.5	3.3	3.0
		C	28.1	1.6	4.6	57.0	8.7	D	0.9	10.0	29.4	53.2	-	3.3	3.2
	A21	D	23.2	1.8	6.4	60.9	7.8	C	0.5	8.2	35.1	48.3	0.4	3.8	3.6
		C	25.9	1.7	6.0	57.9	8.5	D	1.2	10.0	30.4	50.8	0.1	4.3	3.3
	A24	D	22.5	1.4	4.8	62.8	8.6	C	0.5	8.5	37.1	46.2	0.5	3.3	3.9
		C	25.8	2.3	5.4	58.8	7.7	D	0.8	9.5	30.8	50.5	-	3.8	4.7
	spf358	D	26.9	2.0	5.7	55.2	10.1	C	1.0	10.5	34.9	47.0	0.4	3.5	2.6
		C	28.5	1.5	5.4	55.6	9.0	D	1.0	9.8	28.8	53.3	-	4.0	3.1
	avg. diff.		-1.9	-0.1	0.0	1.6	0.5		-0.2	-0.7	6.4	-5.1	0.4	-0.7	-0.1
	std. dev.		0.8	0.5	0.5	1.6	0.6		0.2	0.7	1.3	1.4	0.2	0.5	1.1
High $C_{18:0}$	5	D	18.2	5.9	5.3	61.2	9.5	C	0.8	10.0	37.3	36.3	1.6	2.6	11.5
		C	19.6	5.7	5.0	60.6	9.1	D	0.9	11.8	33.6	39.1	0.9	3.1	10.6

Table 7. (continued)

	6	D	15.2	7.6	9.8	58.9	8.5	C	0.7	10.8	40.6	34.5	1.1	3.7	8.6
		C	16.1	6.5	8.3	61.3	7.7	D	0.8	10.5	33.4	38.1	0.8	4.2	12.2
	7	D	17.5	8.3	6.4	60.2	7.6	C	0.7	10.1	40.6	31.6	1.8	2.3	12.9
		C	16.6	7.6	6.0	61.6	8.1	D	0.9	10.7	34.1	35.5	1.0	3.6	14.2
	8	D	16.0	8.3	6.8	57.3	11.7	C	0.9	10.8	36.9	31.7	2.3	2.9	14.4
		C	17.0	7.6	6.5	60.1	8.8	D	1.1	11.1	33.4	35.0	1.1	4.2	14.1
	stea	D	14.3	9.7	8.7	60.3	7.0	C	0.5	8.4	37.0	33.1	1.9	3.3	15.8
		C	15.8	9.1	7.6	60.2	7.2	D	1.1	11.0	40.8	27.8	2.5	3.2	13.7
	avg. diff.		-0.8	0.7	0.7	-1.2	0.7		-0.2	-1.0	3.4	-1.7	0.5	-0.7	-0.3
	std. dev.		1.0	0.3	0.5	1.5	1.3		0.2	1.2	4.3	3.9	0.7	0.6	2.2
High $C_{16:0}$ & $C_{18:0}$	453	D	21.9	6.8	5.5	55.4	10.5	C	1.3	12.1	36.0	38.1	1.5	3.1	7.8
		C	23.0	4.8	4.8	56.5	10.9	D	0.8	9.6	31.4	35.7	1.0	4.5	17.2
	561	D	21.3	6.0	2.7	59.2	10.8	C	1.2	12.6	39.3	35.8	1.6	1.5	8.1
		C	23.1	5.4	2.8	58.4	10.3	D	1.2	12.2	31.3	42.2	0.9	2.3	9.9
	avg. diff.		-1.4	1.3	0.3	-0.1	0.1		0.3	1.5	6.3	-2.0	0.6	-1.1	-5.6
	std. dev.		0.5	1.0	0.6	1.4	0.6		0.4	1.5	2.3	6.2	0.1	0.3	5.3
Low $C_{16:0}$	10	D	11.9	7.3	14.8	58.6	7.5	C	0.5	9.0	38.2	34.7	1.4	3.9	12.3
		C	12.4	5.6	12.2	61.5	8.4	D	1.2	11.3	34.7	35.9	1.0	5.6	10.2
	spf457	D	13.0	5.6	9.2	68.9	3.5	C	0.1	4.8	45.6	36.3	0.5	3.1	9.5
		C	13.7	4.6	9.0	68.6	4.1	D	0.7	5.8	42.6	38.9	-	2.7	9.2
	spe153	D	12.9	4.6	18.3	56.3	8.0	C	0.7	9.3	35.4	40.7	0.9	6.3	6.7
		C	14.5	3.8	13.8	60.4	7.5	D	1.0	10.4	33.3	41.4	-	6.3	7.6
	avg. diff.		-0.9	1.2	2.4	-2.2	-0.3		-0.5	-1.5	2.9	-1.5	0.6	-0.4	0.5
	std. dev.		0.6	0.5	2.2	2.3	0.7		0.2	0.8	0.7	1.0	0.2	1.1	1.5
Low $C_{18:3}$	A5	D	19.7	2.6	10.9	63.2	3.6	C	0.1	4.3	40.9	44.8	0.2	5.5	4.1
		C	20.2	2.9	10.0	63.5	3.4	D	0.0	5.3	35.5	48.0	-	5.4	5.7
	spb201	D	19.4	2.9	12.6	62.4	2.6	C	0.1	3.3	37.4	47.2	0.2	6.7	5.1
		C	21.3	2.7	12.2	60.5	3.3	D	0.6	4.3	32.1	49.4	-	8.2	5.4
	avg. diff.		-1.2	0.0	0.6	0.8	-0.3		-0.2	-1.0	5.3	-2.6	0.2	-0.7	-1.0
	std. dev.		1.0	0.3	0.3	1.5	0.6		0.4	0.0	0.1	0.7	0.0	1.1	0.9

Table 8. Correlation of PL fatty acid percentages

		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
$C_{16:0}$	PC	1.000	***-0.514***	***-0.526***	-0.339	0.330
	PE	1.000	***-0.736***	***-0.621***	-0.251	0.219
$C_{18:0}$	PC	-	1.000	-0.004	-0.363	0.270
	PE	-	1.000	0.149	-0.105	0.240
$C_{18:1}$	PC	-	-	1.000	-0.039	***-0.429***
	PE	-	-	1.000	-0.097	-0.376
$C_{18:2}$	PC	-	-	-	1.000	***-0.690***
	PE	-	-	-	1.000	***-0.736***
$C_{18:3}$	PC	-	-	-	-	1.000
	PE	-	-	-	-	1.000

Numbers in bold and italic are significant correlation coefficients at 5% level

Table 9. Correlation among PL fatty acid composition and molecular fraction percentage

		18:3/18:3	18:2/18:3	16:0/18:3 + 18:2/18:2	16:0/18:2 + 18:1/18:2	18:0/18:3	16:0/18:1	18:0/18:2
$C_{16:0}$	PC	0.282	0.094	***-0.471***	***0.757***	-0.407	0.287	***-0.565***
	PE	-0.214	-0.137	***-0.730***	***0.803***	***-0.450***	-0.036	***-0.617***
$C_{18:0}$	PC	0.345	0.261	-0.313	***-0.882***	***0.948***	-0.191	***0.988***
	PE	***0.419***	***0.492***	***0.536***	***-0.972***	***0.853***	-0.225	***0.934***
$C_{18:1}$	PC	-0.309	-0.280	0.172	-0.007	-0.062	0.408	-0.013
	PE	0.013	-0.156	0.213	-0.213	-0.094	***0.679***	0.019
$C_{18:2}$	PC	***-0.731***	***-0.504***	***0.899***	0.002	***-0.489***	***-0.492***	-0.297
	PE	***-0.452***	***-0.580***	***0.665***	0.003	-0.168	-0.350	-0.105
$C_{18:3}$	PC	***0.867***	***0.868***	***-0.647***	-0.137	0.422	0.044	0.209
	PE	***0.600***	***0.830***	***-0.502***	-0.152	0.319	-0.223	0.286

Numbers in bold and italic are significant correlation coefficients at 5% level

CHAPTER 6. GENERAL CONCLUSIONS

Soybeans with a wide range of oil compositions were analyzed for fatty acid composition, fatty acid stereospecific distribution, and molecular fraction composition of their major phospholipids (PLs). The physiological performance of the soybean seed with elevated saturated fatty acid percentages were evaluated and correlated with their PL saturated fatty acid percentages. Thermal phase transition temperatures of neutral and polar lipids of modified soybeans were determined to understand how the chemical composition of soybean lipids affect their physical properties and consequently physiological function.

PLs were a minor component of soybean seed, comprising an average of 0.9 % of the seed, which was a equivalent of 3.7 % of the crude soybean oil. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) were the major PL classes in soybeans, with relative proportions of 55.3, 26.3, and 18.4%, respectively. These proportions were affected by the changes in oil fatty acid composition. The fatty acid composition of PLs changed with oil fatty acid modification, especially when the percentages of palmitate, stearate, and linolenate were modified. Stereospecific analysis showed that saturated fatty acids were primarily located at the *sn*-1 position of all PLs, and changes in the saturates of PLs were largely reflected on this position. Oleate was distributed relatively equally between the *sn*-1 and *sn*-2 positions. Linoleate was much more concentrated on *sn*-2 than on *sn*-1 position for all PLs. Linolenate was distributed relatively equally at low concentration but preferred *sn*-2 position at high concentration.

Because PLs are the major component of the cell membrane, their compositional change may relate to the unpredictable and poor seed performance. Soybean seeds with

elevated and reduced percentages of palmitate and elevated percentages of stearate were compared with those of seeds of typical-composition in tests for germination, seedling growth rate (SGR), and leachate conductivity. In general, the seeds with altered compositions did well in these laboratory physiological tests, but their vigor tended to be negatively correlated with percentages of stearate, palmitate or total saturates in various lipid classes. Soybean lines with elevated stearate percentages seemed to perform worse in SGR test than that with elevated palmitate percentages.

The poor seed performance associated with elevated saturate percentages may be attributed to altered PL physical properties caused by compositional changes. Neutral and polar lipids of modified soybeans were evaluated for their phase transition temperatures by differential scanning calorimetry (DSC). Neutral lipids with elevated stearate and both elevated stearate and palmitate had significantly higher melting temperatures than those with elevated palmitate, typical composition, and reduced saturates. PC, the major PL in soybean, from the elevated stearate lines and lines with elevated total saturates had significantly higher phase transition temperatures than the other samples. Increased neutral lipid saturation may make lipids relatively unavailable as an energy source during seed germination at temperatures below its melting point. Increased PL phase transition temperature may cause cell membrane structural defects that affect permeability and enzymatic activities.

To understand the detailed PL structural change caused by oil composition modifications, PC and PE from the modified soybeans were partially fractionated into their molecular species by high-performance liquid chromatography (HPLC). An evaporative light-scattering detector was used for quantification after its non-linear response was corrected. As expected, genetic modification of soybean oil caused changes in PL

molecular fraction composition, and this may have more important implications to the physical properties and physiological functions of phospholipid biomembranes than the other compositional information.

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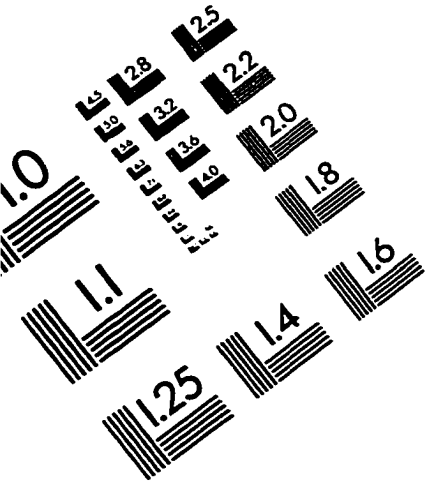
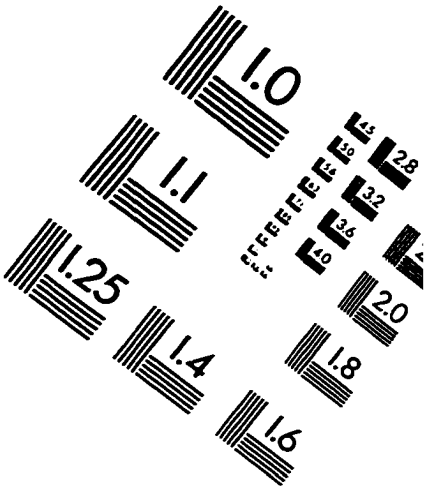
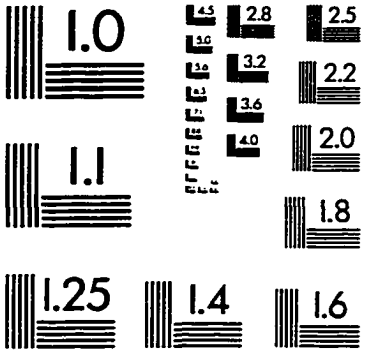
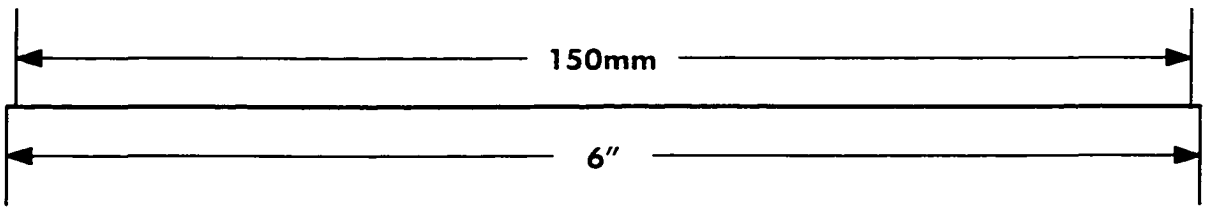
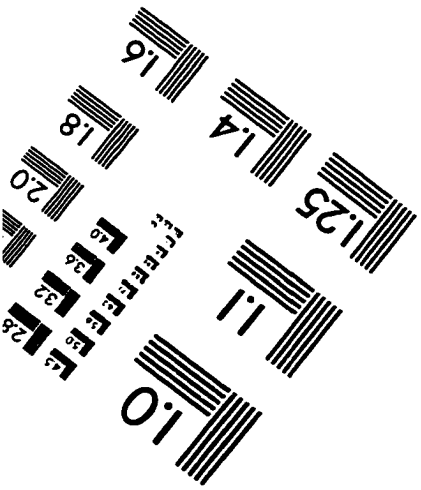
IMAGE EVALUATION
TEST TARGET (QA-3)

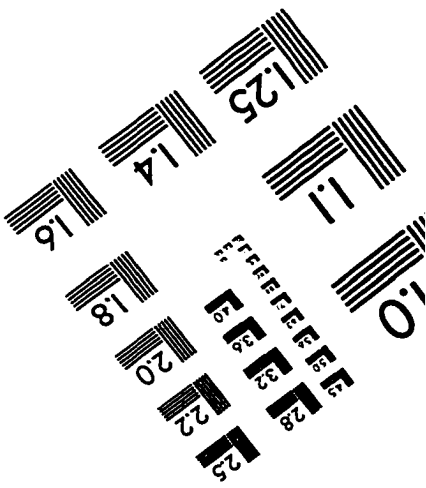












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