Phospholipid Class and FA Compositions of Modified Soybeans Processed with Two Extraction Methods

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ABSTRACT: Soybean lecithin is used as an emulsifier in food, cosmetic, and pharmaceutical industries. The proportion of individual phospholipids (PL) and their FA composition may affect the functional properties of lecithin. In this research, lecithins recovered from four modified soybeans and one commodity soybean, which were processed by extrusion-expelling and conventional solvent extraction, were analyzed for proportion of PL class and FA composition. HPLC with an ELSD analysis demonstrated that the percentage of PC in extrusion-expelled lecithin was higher than in solvent-extracted lecithin, whereas the PE content was lower. GC analysis showed that FA compositions of the PL varied with soybean type. The oil extraction method did not significantly affect FA composition. Critical micelle concentration tested with a tensiometer showed differences among the lecithins.

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

Soy lecithin, a general term for total soybean phospholipids (PL), is widely used in the food, cosmetic, and pharmaceutical industries as an emulsifier, lubricant, and release agent (1). It is a by-product of soybean oil processing. During degumming, water is mixed into soybean oil, and PL hydrate and settle out as gum. Thus, physical stability of the degummed oil is improved and lecithin is produced by further drying of the gum. PL in soy lecithin are approximately 55.3% PC, 26.3% PE, and 18.4% PI (2). Each of these phosphatides has two fatty acyl chains and a polar head group, which give the surfactant characteristics of lecithin. Owing to the difference in their head groups, the three PL have different functional characters; thus, functional properties of the lecithin product may be different if the relative proportion of these three PL changes (3).

Soybean oil can be extracted by two methods: mechanical pressing, such as extrusion-expelling (E-E), and solvent extraction (SE). SE processing uses hexane and is a common practice for conventional oil processing plants (typically 3000 metric tons feed/d) (4). E-E technology uses a dry autogenous extruder that generates pressure and heat that disrupt the cellular structure of the seed. A screw press is then used to press the oil out. E-E plants are relatively inexpensive to construct and operate, and value-added soybean products may be produced. Farmers in North America are increasingly building E-E facilities (74 plants in the Midwest soybean-producing area as of spring 1999) (4). Different oil extraction methods affect the minor component profile of oil; for example, E-E crude oil contains low levels of PL and FFA (4–7). It was also reported that the PL class profile of lecithin could be changed by different oil extraction methods; for example, lecithin from the expander process contains more PC and less PE than SE lecithin (8).

Soybeans can be genetically modified either to obtain an improved oxidative stability of oil [e.g., high-oleate (HO), low-linolenate (LLL), and lipoxygenase-free (LOX) soybeans] or to obtain highly nutritional oil, such as the low-saturates (LS) soybean. Oilseed modification causes the change of FA profiles of PL (3), and this change may in turn affect the functional properties of lecithin, such as emulsification properties and oxidative stability (2). Therefore, it is important to determine the PL class and FA compositions of the PL of soy lecithin.

The objective of this research was to determine whether and how the soybean lecithins from the two oil processing methods, as well as from the genetically modified soybean seeds, were different regarding their PL class and FA compositions. Lecithins from four types of genetically modified soybeans and one from commodity soybeans processed with both E-E and SE processing were analyzed for their PL class profile and FA composition in each PL class. The critical micelle concentration (CMC) was also determined to examine the functionality of soy lecithin. The PL molecule contains both the lipophilic fatty acyl group and the hydrophilic head group, and this feature gives it surface-tension reduction capability, which could be quantified by CMC. Above CMC, the thermodynamic activity of the emulsifier does not increase with the addition of more emulsifier (9). A low CMC value indicates better emulsification capability (9).

EXPERIMENTAL PROCEDURES

Soybean seed sources. Commodity soybeans (CS) were obtained from West Central Cooperative (Ralston, IA) and were used for comparing the PL class proportions and FA profiles of individual PL with other genetically modified soybeans. Three of the soybeans with modified FA compositions were obtained from Optimum Quality Grains (Des Moines, IA): an HO line, A2333HO, containing 79.2% oleate; an LS line,

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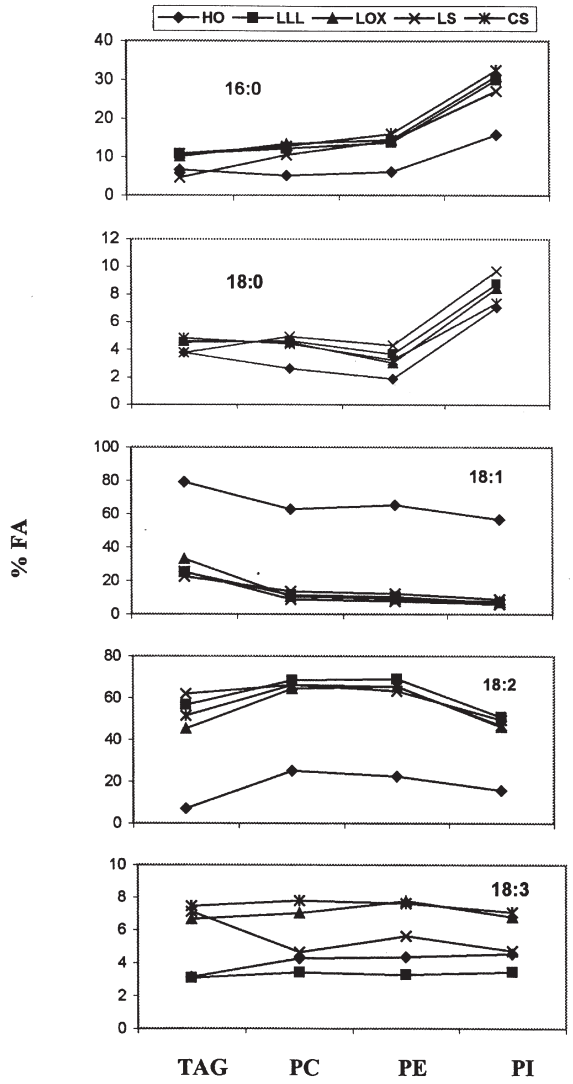

FIG. 1. FA percentages of TAG and phospholipid (PL) classes in five types of soybean: CS, commodity soybean; LLL, low-linolenate soybean; LOX, lipoxygenase-free soybean; HO, high-oleate soybean; LS, low-saturates soybean.

P92B72, containing 8.4% total saturated FA; and an LLL line, P9322, containing 3.1% linolenate. An LOX line, IA2027, provided by the Committee for Agricultural Development, Iowa State University (Ames, IA), was also used. FA composition of these seeds is illustrated in Figure 1.

Soybean processing. (i) E-E processing. Five types of soybeans (20 bu, or 540 kg each) were processed at a commercial E-E plant (formerly Iowa Soy Specialties; now Nutriant, Vinton, IA) by using an Insta-Pro extruder (Model 2500) and screw press (Model 1000). The seeds were cracked with a roller mill and dehulled by aspirating, and the meals were extruded and expelled. The meals and oils were collected in an identity-preserving fashion after the residuals from the previous seed lot were flushed and operating parameters restabilized.

(ii) SE processing. A pilot plant-scale solvent extractor simulator (French Oil Mill Machinery Co., Piqua, OH) was used to extract one bushel (27 kg) of dehulled and flaked soybeans. Five stages of hexane extraction were used at 60°C. The majority of the hexane was then evaporated with desolventizer, and the residual hexane was removed with a laboratory rotary evaporator at 65°C.

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

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

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

PL class separation and quantification with HPLC. A Beckman Coulter (Fullerton, CA) HPLC system with auto sampler 508, solvent delivery module 126, silica column (250 mm length, 2.1 mm i.d., from Alltech), and an ELSD 2000 (Alltech) was used for the PL class composition analysis. Two solvent mixtures were used: *A* was chloroform/methanol/ammonium hydroxide (80:19.5:0.5, by vol), *B* was chloroform/methanol/water/ammonium hydroxide (60:34:5.5:0.5, by vol). Flow rate was 0.3 mL/min. Nitrogen gas at a flow rate of 1.6 L/min was used to evaporate the solvent in the heated chamber at 50°C. The gradient program of these two mobile phases is shown in Table 1.

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

Statistical data analysis. Data were analyzed with the General Linear Model of the SAS program (12). A factorial experimental design was used to examine the effects of soybean

TABLE 1
Gradient Program of Mobile Phase in HPLC Analysis of PL[a]

Time (min)	Solvent A[b] (%)	Solvent B[c] (%)
0	100	0
5	45	55
10	30	70
15	30	70
20	0	100
25	0	100
30	50	50
35	100	0

[a]PL, phospholipid.
[b]Solvent A: chloroform/methanol/ammonium hydroxide = 80:19.5:0.5 by vol.
[c]Solvent B: chloroform/methanol/water/ammonium hydroxide = 60:34:5.5:0.5 by vol.

seed type (five seeds) and extraction method (two methods) on FA and PL class compositions. The least significant differences (LSD) at $P = 0.05$ were calculated to compare treatment differences. Each processing and analysis method was duplicated.

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

RESULTS AND DISCUSSION

FA composition. Statistical analysis demonstrated that there was no interaction between extraction method and soybean seed type for FA composition. Generally, extraction methods did not significantly affect the FA composition of the PL classes, as shown in Table 2, so the average percentages of FA in individual PL of E-E and SE lecithins could be used for comparing the seeds. FA compositions of TAG from the five soybean seeds were also included in Figure 1 to compare the difference in composition between TAG and PL.

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

FA profiles of TAG and PL were also different. The palmitate and stearate contents of TAG were similar to those of PC and PE but different from those of PI, with PI containing much more of the two saturated FA than did TAG, PC, and PE. The oleate content of TAG was about 20% higher than that of all three PL, whereas the linoleate content of TAG was lower compared with that of the PL. The linolenate contents of TAG and the PL were not significantly different.

TABLE 2
$LSD_{0.05}$ and *P* Values for the Effect of Extraction Method and Soybean Seed Type on FA Profile[a]

			Palmitate	Stearate	Oleate	Linoleate	Linolenate
PC	Processing method[b]	$LSD_{0.05}$	2.20	0.70	3.61	3.90	0.75
		P value	0.7517	0.0023	0.4219	0.3265	0.0012
	Soybean seed type[c]	$LSD_{0.05}$	1.39	0.44	2.29	2.46	0.48
		P value	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
PE	Processing method	$LSD_{0.05}$	1.37	0.58	1.50	1.72	0.06
		P value	0.3702	0.0523	0.1921	0.14	0.0102
	Soybean seed type	$LSD_{0.05}$	2.16	0.92	2.37	2.72	0.98
		P value	<0.0001	0.0017	<0.0001	<0.0001	<0.0001
PI	Processing method	$LSD_{0.05}$	1.83	1.08	1.60	1.62	0.79
		P value	0.1557	0.015	0.9545	0.9435	0.8397
	Soybean seed type	$LSD_{0.05}$	2.87	1.71	2.53	2.57	1.26
		P value	<0.0001	0.0334	<0.0001	<0.0001	0.0003

[a]E-E, extrusion-expelled; SE, solvent extraction; CS, commodity soybeans; LLL, low-linolenate soybeans; LOX, lipoxygenase-free soybeans; HO, high-oleate soybeans; LS, low-saturates soybeans.
[b]Processing method refers to soybean oil extraction methods: E-E and SE.
[c]Soybean seed types refer to the five genetically modified soybean seeds: CS, LLL, LOX, HO, and LS.

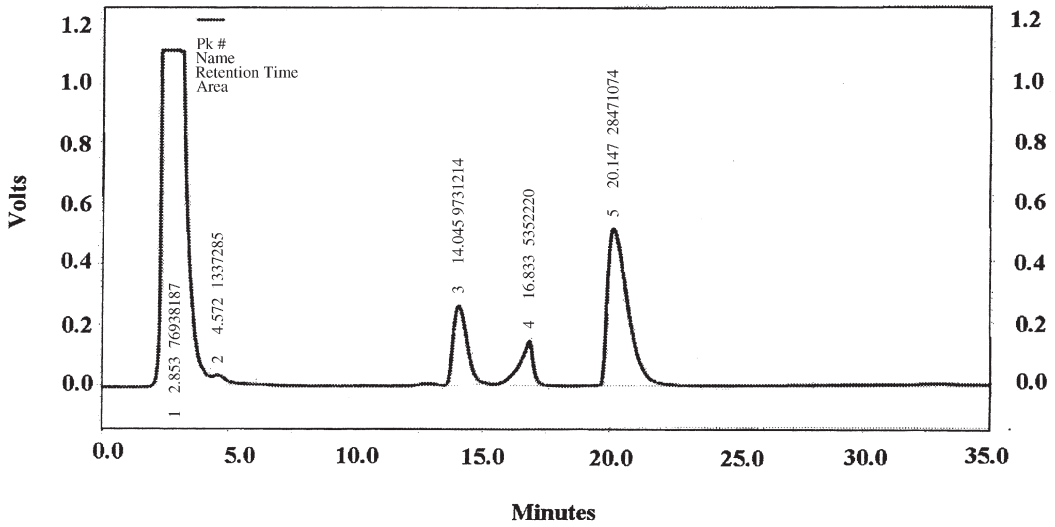

FIG. 2. HPLC chromatogram of a PL sample. Peaks 3, 4, and 5 are PE, PI, and PC, respectively. For abbreviations see Figure 1.

Comparison of the three PL showed that PI contained more saturated and less unsaturated FA than did PC and PE. This characteristic of PI and its unique head group structure may give PI different properties from PC and PE, such as the different solubility in ethanol, which is the basis of fractionation of PC and PI.

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

$$\text{PC: } Y = 10^6X + 680{,}998 \ (R^2 = 0.9912) \quad [1]$$

$$\text{PE: } Y = 909{,}079X^{1.228} \ (R^2 = 0.9985) \quad [2]$$

$$\text{PI: } Y = 452{,}779X^{1.339} \ (R^2 = 0.9995) \quad [3]$$

Melton (13) reported linear calibration curves for PE (10–150 μg), PC (10–250 μg), and PI (10–75 μg); Balazs *et al.* (14) reported linear calibration curves for all three PL for 2–8 μg PL per injection, whereas Christie (15) claimed nonlinear relationships for all of them for injections of 1–5 μg. In our injection range, a linear result was obtained for PC (1.25–25 μg) and nonlinear results for PE (0.5–25 μg) and PI (0.25–10 μg). It is obvious that the injection ranges of the above are quite different. It was reported that linear response of the ELSD decreased drastically below 10 μg PL (13). Injection of PL at higher amounts appears to result in a linear response

TABLE 3
Average PL Percentages of the Acetone De-oiled Soybean Lecithins Recovered from E-E and SE Processing[a]

Extraction method	Soybean seed type				
	CS	LLL	LOX	HO	LS
E-E	24.69	17.01	21.04	22.68	40.84
SE	29.26	15.24	22.97	22.77	17.51

[a]For abbreviations see Table 2.

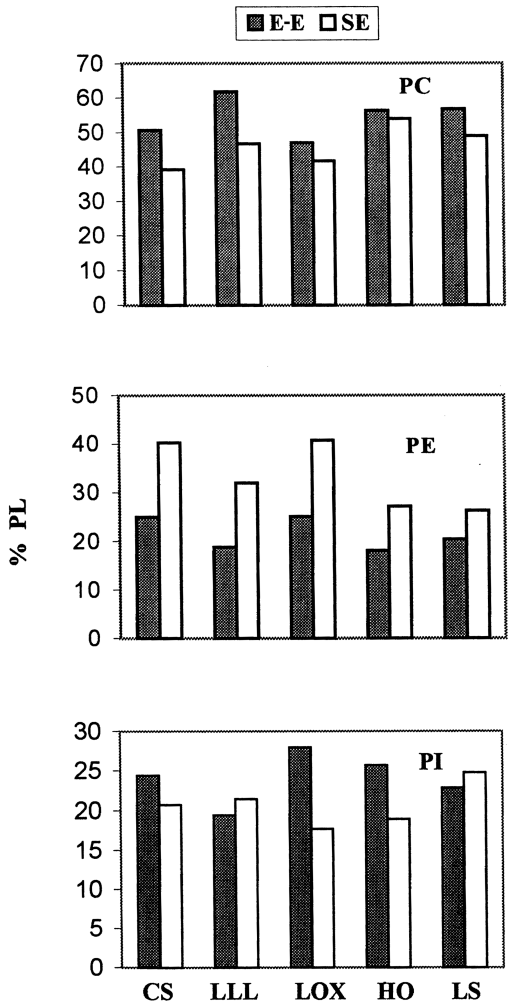

FIG. 3. PL percentages of lecithins from E-E and SE processing of five types of soybeans: CS, LLL, LOX, HO, and LS. E-E, extrusion-expelled; SE, solvent extraction; for other abbreviations see Figure 1.

TABLE 4
$LSD_{0.05}$ and *P* Values for the Effect of Extraction Method and Soybean Seed Type on PL Class Composition[a]

		PC	PE	PI
Processing method[b]	$LSD_{0.05}$	2.20	0.70	3.61
	P value	<0.0001	<0.0001	0.0028
Soybean seed type[c]	$LSD_{0.05}$	1.39	0.44	2.29
	P value	<0.0001	<0.0001	0.0735

[a]For abbreviations see Tables 1 and 2.
[b]Extraction methods refer to soybean oil extraction methods: E-E and SE.
[c]Soybean seed types refer to the five genetically modified soybean seeds: CS, LLL, LOX, HO, and LS.

in the detector. This is in agreement with our results, as shown above, that the PC calibration curve, which started from a higher injection amount than PE and PI, resulted in a linear relationship between injection amount and detection response, whereas PE and PI did not. We used the higher starting injection amount of standard PC because the PC contents in our samples were higher than PE and PI, so the lower range of injection was not necessary. It was also reported that the linear range depended on the specific commercial model of the detector and that it actually depended mostly on the nebulizer design (15,16). In our study, PL classes were well separated (Fig. 2), and a smooth baseline and good reproducibility were obtained, except that retention times for the three PL became closer to each other after continuous analysis owing to the water-containing mobile phase. Balazs *et al.* (14) also reported that equilibrating silica columns was difficult when water was present in the mobile phase. The retention time for PE ranged from 12.4 to14 min, for PI from 16.5 to 17.5 min, and for PC from 18.5 to 20.2 min.

Contents of each individual PL class were quantified using the above standard curves. Total amounts of PL in the 10 samples are shown in Table 3. The purity of the lecithins was still very low although they were de-oiled with acetone. This corresponds with our observation that the de-oiled lecithins appeared very oily. A considerable amount of oil apparently remained in the lecithin. To obtain purer lecithin, multiple acetone precipitations may be needed. Table 3 also shows that LS lecithins from E-E and SE processing were quite different; this may be due to experimental errors, such as nonuniform sampling or inconsistent de-oiling.

The relative proportions of PL classes for five soybean seeds and two extraction methods are presented in Figure 3. Statistical analysis showed that extraction method significantly affected PL composition, and there was significant interaction between extraction method and soybean seed type. E-E lecithin contained significantly more PC and less PE than SE lecithin. E-E processing may result in a superior PL profile of lecithin. It should be noted that lecithins enriched in PC are considered better hydrophilic emulsifiers in cosmetic and pharmaceutical products (17). In addition, E-E oil is processed mechanically without any organic solvent treatment, so it is more appealing to consumers who prefer natural foods or goods.

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

TABLE 5
CMC (mg/mL) of Lecithins from Five Types of Soybeans Processed with E-E and SE[a]

Processing method	Soybean seed type				
	CS	LLL	LOX	HO	LS
E-E	1.34	1.63	1.75	2.5	4.13
SE	4.04	1.87	5.45	1.18	3.63

[a]CMC, critical micelle concentration; for other abbreviations see Table 2.

Statistical analysis also showed that soybean seed type significantly affected the PL composition (Table 4). PC and PE contents of CS and LOX lecithin obtained from both E-E and SE extractions were similar for two of the seeds, but they were significantly different from those of the other three seeds. Apparently, the genetic modification of LOX did not affect relative PC and PE contents, probably because the LOX seeds were modified to remove only the lipoxygenases, which are the enzymes for lipid oxidation. PC contents of LLL, HO, and LS from both E-E and SE lecithins were all higher than E-E and SE lecithins from CS, whereas PE contents were all lower. It appears that genetic modification of certain seeds would not only improve the oxidative stability of their oils but also increase the PC content in their lecithins, adding value to their lecithin products.

CMC determination of the lecithins. The CMC of the 10 lecithins were determined and are presented in Table 5. Statistically, there were no correlations between CMC and composition of individual PL class and FA ($P > 0.05$). Owing to the high impurity content of our samples and the number of sample types, it is difficult to correlate the CMC directly with single factors. Certain trends may be obtained from plotting CMC with PL class composition. CMC decreased as the proportions of PC and PI increased, with the slope of PI being greater than that of PC. This may indicate that a change in the proportion of PI could make more drastic changes in CMC than a change in PC.

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