Total and Polar Lipids in Soybean Protein Meals

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ABSTRACT: Soybean protein meals obtained by various oil extraction methods have different neutral oil content, and they may contain different amounts of polar lipids. Three soy protein meals obtained by different processing methods were extracted by two solvents consecutively, chloroform/methanol (2:1, vol/vol) and water-saturated butanol, for total lipid analysis. The organic flour (i.e., ground soybean) contained 15.52% total lipids; the high protein dispersibility index flour from extrusion-expelling processing and the white flour from conventional solvent extraction contained 11.20 and 1.84% total lipids, respectively. Organic flour contained more polar lipids than the other two protein meals on a dry-weight meal basis. Chloroform/methanol extracted most of the lipid from the meals, whereas water-saturated butanol resulted in an extract with more polar lipids than that from chloroform/methanol extraction.

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Soybean protein meals or flours obtained after oil extraction still contain certain amounts of neutral and polar lipids/phospholipids (PL). The residual lipid content and composition may be different depending on the method used for processing the soybeans. These protein meals may perform differently in their functional properties (including emulsification capacity, emulsion stability, foaming characteristics, water and oil binding properties, etc.) in food or feed applications because of the differences in their neutral and PL profiles. This study was designed to examine the complete lipid profile of protein meals from different processing methods: conventional solvent extraction (SE), extrusion-expelling (E-E), and fine grinding for full-fat soy flour production. The SE method uses hexanes as solvent to extract oil, and the E-E method uses mechanical pressure to extrude the seed and expel the oil.

Total lipid quantification is affected by the solvent used for extraction. Two solvents, chloroform/methanol (2:1, vol/vol) and water-saturated butanol, can be used consecutively to extract lipid from the protein meals to ensure complete extraction. Water-saturated butanol has higher polarity than chloroform/methanol, and it is expected to extract any residual PL from the protein meal. A quantitative comparison of total lipids and PL in these extracts is important so that certain modifications of the routinely used extraction method may be made to accurately quantify various lipid classes.

EXPERIMENTAL PROCEDURES

Materials. A high protein dispersibility index (PDI) soy flour and an organic soy flour were provided by Nutriant (Vinton, IA), a subdivision of Kerry International (Beloit, WI). The high-PDI flour was obtained from E-E processing with moderate protein denaturation. Organic flour was produced by dehulling and grinding the soybeans. White flour was purchased from Archer Daniels Midland (Decatur, IL), and it was produced by conventional solvent extraction and flash desolventization.

Total lipid extraction by two solvents. Samples of about 10 g of ground meal (passed through a 100-mesh sieve) were weighed and dispersed in chloroform/methanol (2:1) at a solvent-to-meal ratio of 5 with constant stirring for about 1 h. The filtrate was washed using a standard Folch *et al.* procedure (1) to remove water-soluble contaminants. Water-saturated butanol was then used at the same solvent-to-meal ratio to extract (1 h stirring) any residual PL. The solvent-free lipid sample was then redissolved in chloroform/methanol (2:1) and a Folch wash was applied to purify the sample.

GC quantification of the extracted lipids. The extracted total lipids were converted to FAME and analyzed by GC for quantification. Heptadecanoic acid methyl ester with a purity greater than 99% (Sigma, St. Louis, MO) was used as an internal standard (2). It was assumed that all lipids in the extract were TAG, so the total lipid quantity was calculated based on FAME quantification. By this assumption, we may underestimate the total lipids by about 6% using an average of 50% PL in total lipids (average M.W. of soybean oil and PL are 874 and 765, respectively). Because the PL content of each lipid sample was different, this assumption simplified the quanification without appreciably affecting the results.

PL quantification. The extracted lipids were dissolved in chloroform/methanol (2:1), and PL content and the PL class proportion were quantified by HPLC according to a method described previously (3).

Statistical analysis. A factorial experimental design was used to examine the effects of product type (three meals) and extraction solvent (two solvents) on total and PL content, as well as the PL class proportion. Treatments were duplicated. Data were analyzed using the General Linear Model of the SAS program (4). The LSD at $P = 0.05$ were calculated to compare treatment differences.

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RESULTS AND DISCUSSION

Comparison of lipid extraction from various meals. Statistical analysis showed that the three meals contained significantly different amount of total lipids and PL (Table 1). Organic meal contained 15.52% of total lipids (as quantified by GC) based on the total of two solvent extracts, which was higher than the total lipids of high-PDI (11.20%) and white flour (1.84%) samples (Table 2). This shows that conventional SE processing (white flour) resulted in much less total lipids left in the meal than did the E-E processing (high-PDI). The total lipid contents of high-PDI, organic, and white flour extracts by chloroform/methanol were 84.76, 61.01, and 52.38%, respectively. Chloroform/methanol is a standard solvent for extracting total lipid material. Its relatively high polarity allowed the extraction of significant amounts of other nonlipid materials that could not be removed by a Folch wash. It seems that the amount of impurities that can be extracted by this solvent is dependent upon the product type. This fact needs to be considered when conducting quantification by a gravimetric method.

The PL contents of the meals were also significantly affected by the meal types (Table 1). Organic meal contained more PL than the other two meals on a dry-weight meal basis (Table 2).

Comparison of lipid extraction by two solvents. When extracted with chloroform/methanol, the high-PDI, organic, and white flour meals had 10.99, 14.71, and 1.78% lipids (dry-weight basis of the protein meal) as quantified by GC. Lipids extracted by water-saturated butanol from the three protein meals were 0.22, 0.80, and 0.07% of the meals, respectively (Table 2). The total lipid contents of chloroform/methanol extracts were higher than those of water-saturated butanol extracts, which were 3.34, 27.44, and 21.54% for high-PDI, organic, and white flour meals, respectively. It suggests that heating or extrusion treatment made the total lipid more extractable by chloroform/methanol than grinding or flaking did. It is obvious that chloroform/methanol extracted most of the lipids, so higher lipid contents in the extracts were obtained. Although chloroform/methanol extracted more PL than water-saturated butanol, the percentages of PL in total lipids extracted by water-saturated butanol were much higher than those extracted by chloroform/methanol except for white flour. The PL contents relative to total lipids extracted by chloroform/methanol for high-PDI, organic, and white flour were 10.10, 9.58, and 70.20%, and those by water-saturated butanol were 50.00, 55.00, and 28.57%, respectively. Therefore, white flour had a higher polar lipid content (relative to total lipids) than did the other two types of protein meal.

Butanol-extracted lipids accounted for only 1.9, 5.2, and 3.8% of the total lipids extracted from high-PDI, organic, and white flours, respectively. However, PL extracted by butanol accounted for 9.0, 23.8, and 1.6% of the total PL extracted from these three protein samples. It seems that the polarity of chloroform/methanol was not high enough to extract total PL from all types of samples. Apparently, PL contained in the organic flour were more tightly associated with the protein. Grinding may not have caused as much cell distortion or disruption as flaking and extrusion; thus, the PL were less extractable with chloroform/methanol.

Comparison of PL class proportions of the three protein meals. The PL class proportions of the lipids extracted by chloroform/methanol and water-saturated butanol from the three protein meals were very different (Table 3). The percentages of PE in the extracts did not change considerably and ranged from 19.65 to 25.76%, whereas those of PI and PC varied considerably with the type of protein meal and extraction solvent. Lipids extracted with chloroform/methanol contained more PC than PI, whereas lipids extracted with water-saturated butanol contained more PI than PC except for the high-PDI sample (Table 3). Overall, the percentages of PI were significantly higher in the butanol extract than in the chloroform/methanol extract. It seems that PI interacted with

TABLE 1
***P* and $LSD_{0.05}$ Values for the Effects of Soybean Meal Type and Solvent on Lipid Extraction and Composition**

Lipid profile	Product type	Solvent	Interaction
Lipid extraction			
Total lipids			
P	<0.0001	<0.0001	<0.0001
$LSD_{0.05}$	0.691	0.567	
Phospholipids			
P	0.0003	<0.0001	0.0814
$LSD_{0.05}$	0.118	0.097	
Phospholipid class composition			
PE			
P	0.1231	<0.0001	0.2061
$LSD_{0.05}$	2.357	1.921	
PI			
P	<0.0001	<0.0001	0.0006
$LSD_{0.05}$	5.146	4.193	
PC			
P	<0.0001	<0.0001	0.0035
$LSD_{0.05}$	5.523	4.500	

TABLE 2
Total Lipid and PL Contents in Soybean Protein Meals[a]

Soybean protein meal	% Total lipid			% PL		
	$CHCl_3$/MeOH	Butanol	Total	$CHCl_3$/MeOH	Butanol	Total
High PDI	10.99	0.22	11.20	1.11	0.11	1.22
Organic	14.71	0.80	15.52	1.41	0.44	1.85
White flour	1.78	0.07	1.84	1.25	0.02	1.26

[a]Quantification was on a dry-weight basis. PDI, protein dispersibility index; PL, polar lipid/phospholipid.

TABLE 3
PL Class Composition of the Lipids Extracted with Chloroform/Methanol and Water-Saturated Butanol[a]

Soybean protein meal	$CHCl_3$/MeOH			Butanol		
	PE (%)	PI (%)	PC (%)	PE (%)	PI (%)	PC (%)
High PDI	25.77	10.01	64.22	22.17	29.85	47.99
Organic	25.39	13.56	61.06	17.89	58.80	23.31
White flour	24.49	24.43	51.08	19.65	58.23	22.12

[a]Quantification was on a dry-weight basis. PDI, protein dispersibility index; PL, polar lipid.

protein more closely than did PC, so it needed a more polar solvent (i.e., water-saturated butanol) to disrupt its interaction and to facilitate its extraction.

The high-PDI sample had significantly lower percentages of PI in its extracted lipids than did the other samples. This may be explained by the nature of the seed processing method. In the E-E process, the seeds are extruded or expanded, during which the interaction among various seed components may be strengthened. Therefore, the E-E protein meal may contain more unextractable PI than other types of protein meal. Currently, many solvent extraction plants use expanders for seed preparation to increase throughput, to improve solvent drainage, and to decrease meal fines in the extract. The protein meals obtained this way may have relatively higher PI content than the protein meal obtained by conventional oil extraction directly from flakes. The functional properties of protein concentrates or isolates prepared from these different types of protein meals may be influenced by such a PL composition shift.

The intention of this study was to verify the hypotheses that total lipid and PL profiles are different in different types of soybean meals and that the lipid extraction method can affect such quantification. More work is needed to study the functional properties of these protein meals and to relate the lipid composition data to meal functionalities. Soybeans from the same variety and meals with a similar degree of protein denaturation are required for such study, which was not possible with the samples used for the current study.

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