EFFECT OF BOUND LIPID ON FLAVOR OF PROTEIN ISOLATE FROM CORN GERM

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ABSTRACT

Cereal Chem. 54(3): 503-510

Protein isolated from solvent-extracted dry-milled corn germ contains little free lipid but contains 7–11% bound lipid, the presence of which results in flavors describable as stale and rancid; such flavors reduce the utility of the protein in food systems. Removal of bound lipid improves both flavor and odor of corn germ protein isolate; however, the flavor of the improved product is still not as bland as that of sodium caseinate. The two best methods developed for removing bound lipid were: 1) extract corn germ meal three times with 5 vol of 80% ethanol before protein isolation or 2) vigorously extract the still wet, freshly precipitated protein three times with 10 vol of 80% ethanol.

Preliminary studies have been described on the preparation of a protein isolate from dry-milled corn germ (1). The protein has a good balance of essential amino acids[2] and has functional properties that indicate potential for food uses. Hexane extraction indicates that protein isolate produced from corn germ contains only 0.35% free lipid (1); however, a gas-liquid chromatographic (glc) procedure established that the isolate contains 7–11% lipid, most of which is bound. This lipid is, at least in part, responsible for undesirable flavors which become more pronounced as oxidative rancidity occurs during storage. This paper describes flavor improvements achieved for corn germ protein isolate by extraction of bound lipids and other substances.

MATERIALS AND METHODS

Preparation

Laboratory. Corn germ protein isolate was prepared by modifications of previously described methods (1). The protein was solubilized by agitating the hexane-deoiled, dry-milled corn germ meal in a Waring Blendor vigorously enough to cause a 15°–25° C temperature rise, first with 10 vol of 0.025*M* sodium hydroxide and then with 10 vol of water. The insolubles were separated from the extract each time by centrifugation. Protein was precipitated from the combined extracts by adjusting pH from 8.5 ± 0.3 to 4.7 with 1 M hydrochloric acid; then it was collected by centrifugation, washed with water, adjusted to neutral pH, and freeze-dried.

Corn germ protein was also precipitated from the extracts adjusted to pH 7.0 by adding alcohols, such as methanol, ethanol, or 2-propanol. Alcohol was slowly added to the protein-containing extract to the desired concentration from 20 to 95%. Precipitated protein was removed by centrifugation. Sometimes, more protein was precipitated from the alcohol-containing solution by adjusting

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[2]Protein isolate produced from corn germ has a corrected PER of 2.36, assuming 2.5 for casein (unpublished data).

to pH 4.7.

Protein isolate was also prepared from hexane-deoiled corn germ meal that was extracted batchwise three times at room temperature with 5 vol of 80% ethanol.

Pilot plant. Corn germ protein isolate was also prepared on a small pilot-plant scale, using a Cowles dissolver rather than a Waring Blendor for vigorous agitation, and increasing the sodium hydroxide concentration to 0.05*M*, which resulted in a pH 9.5 ± 0.5 extract. The pilot-plant preparations of corn germ protein isolate were not washed with water before freeze-drying.

Purification

Almost all the solvents used to extract lipids and other undesirable compounds from corn germ protein isolates were similar to those used by Eldridge *et al.* on soy products (2,3).

Generally, laboratory-prepared corn germ protein isolate was extracted three times in a Waring Blendor at low speed with 10 ml of 80% ethanol/g solids at room temperature. In one experiment, the calculated amount of pure ethanol was added to still wet, freshly precipitated isolate, which was slowly blended to yield protein suspended in 80% ethanol. After the protein was adjusted to neutral pH and separated by centrifugation, it was washed two more times with 80% ethanol (10 ml/g). Solvent was removed from the centrifuged protein after the final extraction by freeze-drying.

Germ protein isolate prepared in the pilot plant was used to compare the relative efficiency of the solvents listed in Table I in removing lipid. Each sample was extracted once at 25° C with 10 ml of solvent/g of protein. Agitation was with a magnetic stirrer at about 600 rpm for 1 hr. For 80% ethanol extraction, agitation by stirring for 1 hr was compared with agitation in a Waring Blendor for 5 min.

In some experiments, solids extracted by 80% ethanol were recovered by rotoevaporation under partial vacuum. In two experiments, the recovered ethanol-soluble solids were partitioned between chloroform and water.

TABLE I
Total Lipid Content of Freeze-Dried Pilot-Plant-Prepared Corn Germ Protein Isolate after Extraction with Various Solvent Systems

Solvent System	Agitation Method	Total Lipid %
No extraction	...	10.6
80% Methanol, 20% water	Magnetic stirrer	8.6
80% Ethanol, 20% water	Magnetic stirrer	6.5
80% Ethanol, 20% water	Waring Blendor[a]	5.4
80% 2-Propanol, 20% water	Magnetic stirrer	4.2
Water-saturated 1-butanol	Magnetic stirrer	2.1
20% Methanol, 80% hexane	Magnetic stirrer	4.0
20% Ethanol, 80% hexane	Magnetic stirrer	5.4
20% 2-Propanol, 80% hexane	Magnetic stirrer	8.3
Dry chloroform	Magnetic stirrer	4.9

[a]This sample was also extracted three times with no change in total lipid content.

Analytical Procedures

Protein contents were determined by multiplying Kjeldahl nitrogen values by 5.4 (1); moisture was measured by drying to constant weight at 105°C; ash was determined by adding magnesium nitrate to fix organic phosphate and then heating at 600°C according to AACC Method 08-02 (4); and crude fiber was determined by AACC Method 32-17 (4). The method of Fiske and Subbarow (5) was followed for total phosphorus, and phytate phosphorus was isolated by the method of Pons *et al.* (6). Starch and sugars were measured by the glc procedure of Sloneker (7).

A scaled-down version of a glc procedure described by Black *et al.* (8) was used for the determination of total lipid. In this modification, a sample containing about 10 mg lipid was weighed into a 15-ml serological tube with a Teflon-lined screw cap; to this sample, 3.0 ml of benzene containing 3.0 mg/ml arachidic acid methyl ester as internal standard, 1.0 ml of 10% hydrogen chloride in dry methanol, and 1.0 ml of dimethoxypropane (Eastman) were added. After this mixture was shaken overnight and allowed to settle, 1.0 μl was injected into a 1/8-in. × 6-ft stainless-steel column containing 10% SE 30 on Chromosorb W. Column temperature was 240°C, inlet was 260°C, the flame ionization detector was 260°C, and the helium carrier flow rate was about 35 ml/min. Under these conditions, all the C_{16} acid esters emerged at 2.5 min, all C_{18} at 4.1 min, and the C_{20} internal standard at 8.0 min. Food-grade corn oil served as a standard. Oil content was calculated by:

$$\% \text{ Oil} = \frac{(\text{mg std.})\ (\text{area oil})\ (100)}{(\text{R})\ \ (\text{area std.})\ (\text{mg sample})}$$

in which mg standard is the 9.0 mg of arachidic acid methyl ester internal standard in each sample, area oil is the sum of the areas of the C_{16} and C_{18} methyl ester peaks, and area standard is the area of the C_{20} arachidic acid methyl ester internal standard peak. R, the response factor, is determined for each set of samples by analyzing corn oil (Mazola) and is calculated by:

$$\text{R} = \frac{(\text{mg std.})\ (\text{area oil})}{(\text{mg oil})\ (\text{area std.})}$$

in which mg oil is the amount of corn oil and the other terms are as given above. (A typical value for R is 0.99.) Free lipid was determined by hexane extraction, AACC Method 30-25 (4).

Organoleptic Evaluation

Flavor and odor assessments were determined by a 12-member taste panel trained at the Northern Center to assess soy products (9,10). Panel members were given samples as 2% dispersions in charcoal-filtered tap water and were instructed to describe the predominant odors and flavors in each sample. Each description was assigned a value of 0, 1, 2, or 3 by the panel member, depending on whether that odor or flavor was absent, weak, moderate, or strong, respectively. The listed intensity value (IV) for each odor and flavor is an intensity weighted summation of responses divided by number of panel

members: IV = [(number of weak responses) + 2 (number of moderate responses) + 3 (number of strong responses)]/n, where n is the number of panel members. The IV scale ranged from 0 to 3. Significance of differences between samples was determined by analysis of variance.

Panel members then scored each sample for overall odor and flavor on a 1–10 scale with scores of 1–2 being very strong, 3–4 strong, 5–6 moderate, 7–9 weak, and 10 bland. Listed odor and flavor scores are panel averages, the significance of which was determined by analysis of variance.

A few flavor, odor, and color descriptions are mentioned without scores. These are judgments of the authors.

RESULTS AND DISCUSSION

Lipid Removal with 80% Ethanol

Bound lipid was removed effectively from corn germ protein isolate by washing with 80% ethanol. Removal was best accomplished by adding that amount of ethanol to wet, freshly precipitated protein to make an 80% ethanol suspension, adjusting to neutral pH, vigorously agitating, and then centrifuging. The protein was then washed two more times with 80% ethanol (10 ml/g). Proximate analysis of laboratory-prepared corn germ protein isolate so treated was compared to untreated isolate (Table II). The treatment reduced the total lipid content to 0.15% from 8%, with a corresponding increase in protein content or purity. Total phosphorus content decreased to 0.9 from 1.7%, probably because phospholipids were removed. Since other analytical values were about the same before and after lipid removal, treatment with 80% ethanol apparently removed mostly lipid materials.

Extracting corn germ meal three times with 80% ethanol before isolating protein produces a protein with only 0.15% total lipid; however, this procedure uses more ethanol than if freshly prepared isolate is extracted as described. Laboratory-prepared corn germ protein isolate was also extracted three times with 80% ethanol after being dried. This procedure only reduced total lipid to 0.5–0.7%, compared to the 0.15% achieved by extracting still wet, freshly precipitated isolate.

TABLE II
Proximate Analysis of Laboratory-Prepared Corn Germ Protein Isolate before and after Extraction with 80% Ethanol

Component	Amount in Corn Germ Protein Isolate % (dry basis)	Amount in Ethanol-Washed Corn Germ Protein Isolate % (dry basis)
Protein (N × 5.4)	73	85
Starch	3	3.5
Crude fiber	0.08	0.22
Sugars	0.8	0.0
Total lipid	8	0.15
Free lipid	0.7	0.04
Ash	4.3	4.6
Total phosphorus	1.7	0.9
Phytic acid phosphorus	0.65	0.4

Lipid Removal with Other Solvents

Germ protein isolate prepared in the pilot plant was used to compare the relative efficiency of various solvent systems in removing lipid. This isolate contained less protein, 66 *vs.* 73%, and more total lipid, 10.6 *vs.* 8%, than laboratory-prepared isolate. Because of its higher lipid content and stronger flavor, the pilot-plant preparation was selected to compare effectiveness of lipid removal by aqueous solutions of alcohols from methanol to 1-butanol. Also compared was extraction with hexane containing three different alcohols, as well as agitation by stirring with a magnetic mixer *vs.* agitation in a Waring Blendor.

Amount of lipid in the protein after various treatments is given in Table I. Effectiveness of aqueous alcohol solutions increased, going from 80% methanol to water-saturated 1-butanol. When 20% solutions of alcohols in hexane are compared, methanol-hexane was most effective. Agitation for 5 min in a Waring Blendor removed more lipid than magnetic stirring for 1 hr (solvent 80% ethanol). None of the treatments achieved as low total lipid in the pilot-plant preparation as was achieved by extracting the laboratory preparation three times with 80% ethanol. The difference was not due to one extraction *vs.* three but rather indicated that the pilot-plant preparation bound lipid more firmly than the laboratory preparation. The reason for this firmer binding of lipid is not known.

Material Extracted by 80% Ethanol

The material removed from protein isolate by extraction with 80% ethanol can be recovered by evaporating the alcohol under vacuum. The waxlike material is light brown and can be partitioned into a chloroform-soluble fraction and a water-soluble fraction. The chloroform-soluble fraction was brown and waxlike with a strong burnt waxy odor and a very strong waxy-bitter flavor. The water-soluble portion is dark brown and has a burnt-phenolic odor and a very strong

TABLE III
Total Lipid Content of Laboratory-Prepared Corn Germ Protein Isolate Precipitated by Adding Various Alcohols to Extracts

Alcohol Added	Final Alcohol Concentration %	Final pH	Total Lipid Content of Protein %
Methanol	33	7	6.6
	50	7	5.3
	75	7	2.0
	95	7	0.93
Ethanol	33	4.7	10.9
	50	4.7	11.5
	75	4.7	8.1
2-Propanol	20	7	5.5
	50	7	3.0
	75	7	0.69
	20	4.7	8.1
	50	4.7	8.6
	75	4.7	8.6

bitter-phenolic flavor. The chloroform-soluble fraction contains 50% total lipid, whereas the water-soluble fraction contains 3.1% lipid. Evidently extraction with aqueous alcohol removes other bitter compounds besides products of lipid rancidity.

Corn Germ Isolates Precipitated by Alcohols

Since lipid was precipitated with protein when pH of the alkaline germ extract was adjusted to pH 4.7, precipitation of protein by organic solvents was tested to see if this process would reduce lipid contamination. Such alcohols as methanol, ethanol, or 2-propanol were added to sodium hydroxide extracts of corn germ

TABLE IV
Odor and Flavor Assessments of Laboratory-Prepared Corn Germ Protein Isolates Compared to Sodium Caseinate

Sample	Odor			Flavor		
	Score[a]	Description	Intensity value[b]	Score[a]	Description	Intensity value[b]
Corn germ protein isolate (laboratory-prepared)	6.5	Musty-stale	0.3	4.4	Bitter	1.1
		Cereal-grain	0.5		Astringent	1.0
					Grassy-beany	0.9
					Musty-stale	0.4
					Cereal-grain	0.7
Corn germ protein isolate washed with 80% ethanol (0.15% lipid)	7.3	Cereal-grain	0.3	5.8	Bitter	0.5
					Grassy-beany	0.5
Corn germ protein isolate precipitated from 33% methanol (pH 7)	7.1	Musty-stale	0.5	5.2	Bitter	1.0
		Cereal-grain	0.6		Astringent	1.0
					Grassy-beany	0.5
					Musty-stale	0.4
					Cereal-grain	0.2
Corn germ protein isolate precipitated from 20% 2-propanol (pH 7)	6.3	Grassy-beany	0.4	5.4	Bitter	0.6
		Musty-stale	0.4		Astringent	0.8
		Cereal-grain	0.3		Grassy-beany	0.4
					Musty-stale	0.8
Sodium caseinate	8.7	None prominent		8.1	Bitter	0.3
					Astringent	0.1
					Musty-stale	0.2
					Cereal-grain	0.1

[a]A flavor score of 10 indicates no detectable flavor; 9–7 indicates weak flavor; 6–5 moderate flavor; 4–3 strong flavor, and 2–1 very strong flavor. The same scale applied to odor scores. A full unit difference is significant.

[b]Odor and flavors noted are panel averages based on a scale of 0 as absent, 1 as weak, 2 as moderate, and 3 as strong. A 0.3 unit difference is significant.

adjusted to neutral pH and the alcohols caused most of the protein to precipitate. Amounts precipitated ranged from 40% of the nitrogen in the extract precipitated by adding 33% methanol to 70% of the nitrogen in the extract precipitated by adding 75 or 95% methanol. For comparison, adjusting the pH of the alkaline extracts to pH 4.7 with HCl precipitated 70% of the nitrogen. Most of the nitrogen not precipitated was nonprotein (1). In some cases, additional protein was precipitated by adjusting pH of the alcohol-containing solutions to 4.7. Protein preparations so isolated were nearly white in color.

Total lipid content of various samples of corn germ protein isolated by addition of alcohols is summarized in Table III. Protein preparations precipitated at pH 7 and at 20 or 33% alcohol concentrations contain more total lipid than those precipitated at higher concentrations. The additional protein precipitated by adjusting pH from 7 to 4.7 contains even more lipid. Only two samples in Table III contain less than 1% lipid: corn germ isolate precipitated from 95% methanol at pH 7 and that from 75% 2-propanol at pH 7. Lower total lipid contents can be achieved by using the ethanol washing procedures described above.

Odor and Flavor

Several samples of corn germ protein isolate along with sodium caseinate were evaluated (Table IV). None of the corn germ protein isolate samples was as bland as sodium caseinate. All samples of germ isolate had odor scores in the weak to moderate range. The most common odors noted for the isolate were cereal-grain and musty-stale. The most common flavors noted were bitter, astringent, grassy-beany and musty-stale—off-flavors similar to those found in soy products. These off-flavors can be produced *in vitro* by treating linoleic and linolenic acids with purified lipoxygenase (11). Washing with 80% ethanol significantly improved the flavor score of corn germ protein isolate, and significantly reduced the number and intensity of off-flavors. Isolate had flavor scores in the moderate range when freshly precipitated either from 33% methanol or from 20% 2-propanol. Unfortunately, these two preparations developed rancid flavors upon storage for 6 months at room temperature. The extent of this flavor change was not evaluated by the taste panel.

Acknowledgments

We thank L. T. Black, F. B. Alaksiewicz, and J. H. Sloneker for help in determination of total lipid by gas-liquid chromatography. Certain other analyses were run by C. L. Harlan, C. E. Johnson, and B. R. Heaton. C. Vojnovich and J. Kerr assisted in the pilot-plant preparation of protein isolate from corn germ.

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[Received June 24, 1976. Accepted September 27, 1976]