Lipid-Protein Complexes from Safflower Expeller Cake

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ABSTRACT

Lipid-protein complexes were prepared from safflower prepress expeller cake, which contains about 18% oil and 17% protein, by grinding and extraction with aqueous alkali, then coprecipitation of oil and protein in the extract with acid. In the laboratory, using hammer mills or high-shear homogenizers, complexes were obtained containing up to 48% oil with 46% protein. In the pilot plant, the best extraction, using an attrition mill, yielded a complex containing 44% oil and 47% protein. Phenolic glucosides, which contribute to the bitterness and cathartic activity in safflower meal, were removed during the process. Loaves of bread with 10% of the wheat flour replaced by safflower lipid-protein complexes had acceptable properties and contained 25-36% more protein than the controls.

INTRODUCTION

Safflower is grown primarily for its oil which may be either the regular high-linoleic or the more recently developed oleic type. The seed, which contains about 40% oil, is usually pressed in an expeller, removing about 70% of the oil and leaving an expeller cake containing ca. 18% oil and 17% protein. Hexane extraction of the expeller cake removes the remaining oil and yields a meal which is commonly classified by sieving into high- and low-protein fractions. Both fractions are valuable in animal feeds. But even the high-protein meal is unsuitable for food use, not only because of its fiber content but because it also contains small amounts of phenolic glucosides which make it bitter and mildly cathartic (1). It has been demonstrated that safflower protein isolates with useful nutritional and functional properties can be prepared from safflower expeller cake that has been defatted with hexane at room temperature (2,3). These isolates are almost free of the deleterious glucosides. Oil and protein may be extracted simultaneously from ground oilseeds with aqueous alkali (4). It has now been found that lipid-protein complexes can be prepared directly from the expeller cake and this paper describes attempts to optimize the direct aqueous extraction of the protein and residual oil from safflower expeller cake.

EXPERIMENTAL PROCEDURES

Materials

Regular (high-linoleic) safflower expeller cakes (4 lots with average compositions of 7% moisture, 17% protein, 18% oil, 27% crude fiber, 3.7% ash) were obtained from PVO International, Richmond, CA. Oleic safflower expeller cake (8% moisture, 17% protein, 17% oil, 26% crude fiber, 5.2% ash) was obtained from Agricom International, Grimes, CA. Before use, expeller cakes were granulated through a 0.25-in. screen (Model 43-B granulator, F.J. Stokes Corp.).

Lipid-Protein Complexes

Laboratory preparations. Safflower expeller cake was ground (see following) in 10 times its weight of water and maintained at pH 9 by the addition of 1 N NaOH. The mixture was stirred 30 min, then centrifuged 30 min at 1,200 × g. The supernatant was saved and the residue rinsed with ca. 5 vol of water by stirring 1 hr at pH 9, then centrifuging as above. The fibrous residue was freeze-dried and analyzed. The combined supernatants, emulsions of oil in a protein solution, were acidified to pH 5, the isoelectric point, with 1 N HCl. The oil was coprecipitated with the protein and the resultant lipid-protein complex was separated by centrifugation as just described, adjusted to pH 7 and freeze-dried. Grinders tested were a homogenizer with one pair of fixed and rotating slotted rings, Polytron (BEW-10, Bronwill Scientific), a homogenizer with 3 pairs of slotted rings (Super Dispax, SD45N, G456 generator, Tekmar Co.) and a high-speed (10,500 rpm) hammer mill (Raymond Lab. Mill, 6 in., MS55A, Combustion Engineering Co.). One ground mixture was further homogenized in a Gaulin Lab homogenizer (15M, Gaulin Corp.) operated at 5,000 psi.

Pilot plant preparations. Safflower expeller cake (100-150 lb) was mixed with 10 times its weight of water, maintained at pH 9 with NaOH, and passed 2 times through either a hammer mill (Rietz Disintegrator, RD9, 1/16-in. screen, Rietz Manufacturing Co.) or an 8-in. single disk attrition mill (CE-Bauer, style 148-8) fitted with curved rib plates 8504, 8505. The mixture was stirred 30 min at pH 9, then centrifuged in a P-3000 Super D-Canter (Sharples-Stokes Div., Penwalt Corp.). In some runs, the fibrous cake was reextracted with 5-10 vol of water by stirring 30 min at pH 9, then centrifuging as described. The combined supernatants were adjusted to pH 5 with 2 N HCl and the lipid-protein complex separated in a high-speed sludge-discharging centrifuge (De Laval Separator Co., Model BRPX-2075). The protein complex sludge was adjusted to pH 7-8 with 1 N NaOH, homogenized in a Charlotte Colloid Mill (ND-1, Chemicolloid Labs, Inc.) and then dried either in a spray drier (Bowen Engineering Co., lab. model) or on a double drum drier (24 × 24 in., Buflovac, Buffalo Foundry & Machine Co.).

Analytical and Evaluation Procedures

AOAC procedures (5) were used for proximate analyses. Protein is expressed as N × 5.3 (6).

Specific phenolic glucosides were determined by a thin layer chromatographic method (1).

Baking properties were determined on laboratory pup loaves prepared by a straight dough process used for protein-fortified flours (7). Hydrogenated shortening (3%) was used instead of a dough conditioner. Lipid-protein complexes replaced 10% of the flour and, in one case, the 3% shortening used in the formulation.

RESULTS AND DISCUSSION

Laboratory Preparations

The most critical step in the preparation of these lipid-protein complexes was the grinding of the safflower expeller cake in alkaline water. Table 1 indicates the mills and grinders tested, yields of solids, protein and oil obtained in the lipid-protein complexes and the composition of these complexes. The type of grinding had only small effects on yields of protein but greatly affected yields of oil. Oil yield was doubled when changing from simple stirring to use of a homogenizer with one pair of rings and was increased even further by use of the highest shear homogenizer with 3 pairs of rings. The flow-through homogenizer was not quite as effective, but probably could be made so by increasing the contact time. Extraction of oil and protein was not quite as efficient from the oleic safflower expeller cake.

The best extraction of oil with the high-speed hammer mill was obtained using a fine (1/32-in.) screen. Lower yields of oil were obtained with larger screen openings which reduced residence time in the mill. Tip speed of this mill was about 8,000-16,000 ft/min, which is well over the lower limit of 5,000 ft/min reported for efficient cell disruption and formation of lipid-protein complexes from vegetable materials (8). Extraction of oil was further increased when hammer mill grinding was followed by treatment with a Gaulin homogenizer.

The calculated distribution of oil and protein during processing from safflower seed to lipid-protein complex, based on results from the extraction using a hammer mill with a 1/32-in. screen, is diagrammed in Figure 1. Pressing 100 kg seed in an expeller would yield about 28 kg oil and 72 kg expeller cake containing 12.3 kg oil and 13.8 kg protein. From this could be obtained 40 kg fibrous residue and 21 kg lipid-protein complex containing 8.6 kg oil and 9.7 kg protein. The final acid extract or whey would contain about 0.3 kg oil and 1.0 kg crude protein, most of which consists of nonprotein nitrogen compounds (2).

Pilot Plant Preparation

Two types of mills were tested for grinding the expeller cake in alkaline water on a larger scale, a hammer mill (Rietz disintegrator) and a disc attrition mill. Composition of the lipid-protein complexes and yields obtained from expeller cake are listed in Table II. More efficient extraction of oil was obtained when using the disc attrition mill and yields of oil and protein were improved by a second extraction of the fibrous residue with water at pH 9. The lower yields, compared to those in smaller laboratory equipment (Table I), are partly caused by higher mechanical losses in the larger equipment. Yields from oleic varie-

TABLE I

Safflower Lipid-Protein Complexes—Laboratory

Safflower	Mill or grinder[a]		Yields in complex: Solids % of that in expeller cake	Yields in complex: Protein % of that in expeller cake	Yields in complex: Oil % of that in expeller cake	Composition (mfb): Protein (%)	Composition (mfb): Oil (%)
Regular	None, stirring only		20	68	25	69	22
	Homogenizer	Pairs slotted rings					
↓	Immersion	1	27	74	52	56	35
	↓	3	30	71	68	48	42
Oleic		3	28	67	61	42	41
Regular	Flow-through	3	25	71	56	54	39
↓	Hammer mill, 1/8 in. screen		24	62	49	52	37
	↓ 1/16 ↓ ↓		27	69	57	53	39
	↓ 1/32 ↓ ↓		30	70	70	47	42
↓	Hammer mill, 1/32 in. screen; then Gaulin homog.)		28	63	73	46	48

[a]Description of mills and grinders in Experimental section.

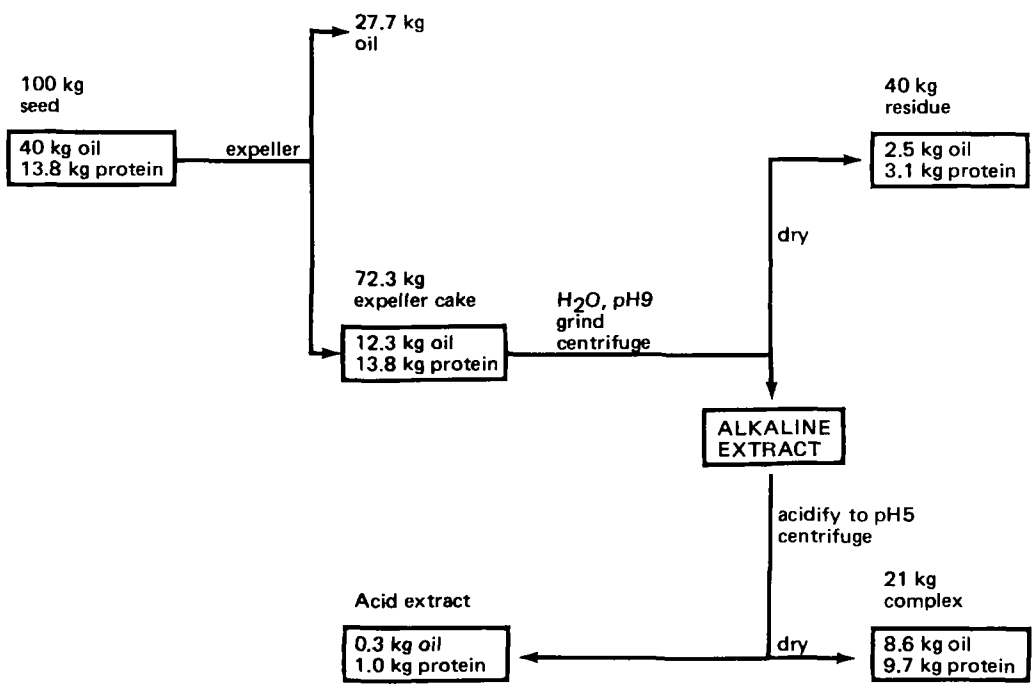

FIG. 1. Distribution of oil and protein in laboratory preparation of safflower lipid-protein complex.

TABLE II

Safflower Lipid-Protein Complexes—Pilot Plant

Safflower	Mill[b]	Extractions	Yields in complex[a] Solids % of that in expeller cake	Protein	Oil	Composition (mfb) Protein (%)	Oil (%)	Fiber (%)	Ash (%)
Regular	Hammer mill	1x	21	68	27	60	25	0.4	1.0
Regular	↓	2x[c]	22	71	35	57	31	0.4	1.2
Oleic		1x	22	65	27	55	22	0.6	3.1
Regular	Disc attrition mill	1x	21	56	49	47	44	0.3	0.8
Regular	Disc attrition mill	2x[c,d]	26	64	54	—	—	—	—

[a]Based on freeze-dried samples.
[b]Description of mills in Experimental section.
[c]Residue from first pH 9 extraction reextracted at pH 9.
[d]Yields calculated from small-scale second extraction.

ties were about the same as those from regular safflower expeller cake.

The percentage recovery of protein, oil and solids at each step of the process was determined in a run using the disc attrition mill and a single alkaline extraction of regular safflower expeller cake. These recoveries in the main protein fractions are plotted in Figure 2. The top curve indicates the total recovery of solids in all fractions, including the fibrous residue and acid extracts; and the distance between this line and 100% recovery is a measure of mechanical losses.

The decrease in recovery going from the expeller cake (at 100%) to the pH 9 extract is a measure of the protein, oil and solids remaining in the fibrous residue. In the same way, the difference in recovery going from the pH 9 extract to the lipid-protein complex is a measure of the material removed in the acid supernatant or whey. In this case, in an attempt to get a cleaner product, the complex was washed twice with water at pH 5, with resultant losses each time. It is evident that the greatest loss occurs during grinding and alkaline extraction and that more oil than protein is lost at this stage.

Evaluation

Protein isolates, prepared from defatted safflower expeller cake, had previously been shown to contain only very small amounts of the phenolic glucosides associated with the bitter taste (matairesinol monoglucoside) and cathartic activity (2-hydroxyarctiin) of safflower meal (1). The concentration of these glucosides found in a safflower lipid-protein complex are shown in Table III, compared to the concentrations in commercial high-protein meal and whole seed meal. Since these glucosides occur only in the seed kernel, their concentrations are highest in the high-protein meal from which most of the hull had been removed. As was expected, most of these water soluble glucosides were removed during the preparation of the lipid-protein complex.

Useful nutritional and functional properties of safflower protein isolates have already been described (2,3). One promising use was as a protein supplement in wheat bread. This seems a good potential use for the lipid-protein complexes, since the safflower oil in the complex might replace some of the shortening or dough conditioner used in bread formulations. These complexes were evaluated as replacements for 10% of the flour in a standard wheat bread (7). The results obtained with pup loaves are shown in Table IV. An overall evaluation score, which is based on several factors listed in the table, is included. Loaf volume is listed separately because it is particularly sensitive to flour replacement. Protein content of the bread was increased 25-36% by the addition of the lipid-protein complexes. Even though the scores and loaf volumes of supplemented breads were somewhat lower than those of the control, satisfactory loaves were obtained from all breads, including a comparative one supplemented with soy flour. Differences between the supplemented breads will be mentioned, even though they are probably not significant. Bread from the first complex (48% protein), when prepared with shortening (3%), was equivalent to that from soy flour. Without shortening, the bread was not quite as good but appeared to be satisfactory. With breads supplemented with the complex containing 62% protein, slightly better results were obtained with the drum-dried complex than with the spray-dried complex. Similar results had been obtained in bread supplemented with a protein concentrate from wheat millrun (7). Complexes from oleic safflower appeared to be as useful as complexes from regular safflower.

Preparation of lipid-protein complexes by aqueous extraction of safflower expeller cake offers the opportunity to obtain a food-grade protein without use of solvent extraction. This process may be particularly advantageous for safflower in which about 70% of the oil is first removed in expellers, separation of the hulls is inefficient and usually avoided, and the deleterious glucosides may be removed by aqueous extraction. Similar processing has been proposed for other oilseeds where it may be advantageous to extract or inactivate deleterious components (9). A more efficient method of grinding the expeller cake in dilute alkali is needed so that less of the oil remains in the fibrous residue.

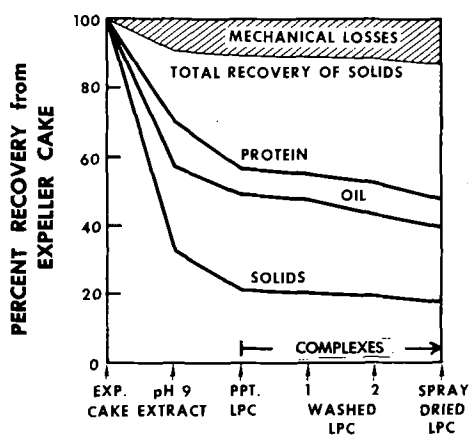

FIG. 2. Recovery of protein, oil and total solids at each step in preparation of a lipid-protein complex (LPC) from safflower expeller cake ground using a disc attrition mill.

TABLE III

Phenolic Glucosides in Safflower Meal and Lipid-Protein Complex

	2-Hydroxyarctiin (%)	Matairesinol monoglucoside (%)
Commercial meal, 42% protein[a]	1.62	0.39
Whole seed meal, 29% protein[a]	0.83	0.18
Lipid-protein complex (50% protein, 42% oil)	0-0.1	0.04

[a]Protein = 6.25 X N.

TABLE IV

Bread Fortified with Safflower Lipid-Protein Complexes (LPC)

Flour replacement (10%)	Protein content (%, mfb)	Loaf vol (mL)	Evaluation score[a]
None (control)	12.2	715	91.0
Soy flour (55% protein)[b]	16.0	605	82.5
Saff. LPC (42% oil, 48% protein)			
with shortening	15.3	610	82.5
without shortening	15.8	590	80.0
Saff. LPC (26% oil, 62% protein)			
spray-dried	16.6	590	81.5
drum dried	16.5	613	82.5
Oleic saff. LPC (23% oil, 61% protein)	16.5	585	81.0

[a]Score based on vol, grain, texture, break and shred, water absorption, mixing time and tolerance, and dough handling properties. Scale 0-100.
[b]ADM Bakers Nutrisoy.

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