NEW TECHNIQUES FOR PREPARATION OF IMPROVED SUNFLOWER PROTEIN CONCENTRATES[1]

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ABSTRACT Cereal Chemistry 53(1): 118-125

Countercurrent extractions of sunflower flour with water, acid, or alcohol, and countercurrent diffusion of sunflower seed with acid, were more efficient in solvent use and chlorogenic acid removal than batch extractions with fresh solvents. With five- to six-stage countercurrent procedures, about 90% of the chlorogenic acid was extracted from sunflower flour at a solvent-to-flour ratio of 6:1 (v/w) or from seed at a solvent-to-seed ratio of 3:1 (v/w). The resulting protein concentrates contained over 70% protein and were light in color under alkaline pH conditions. Acid extraction of the flour produced the most soluble protein concentrate, but 40% of the flour solids and 25% of the flour proteins were lost in the liquor or extract. Acid extraction at 80°C or water extraction of protein-denatured flour improved the rate of chlorogenic acid extraction, but protein losses in the extract remained high. Aqueous ethanol was an efficient solvent for the removal of chlorogenic acid from the flour, and the protein concentrate yield of 77–78% accounted for 95–97% of the flour proteins. Acid diffusion of the seed gave intermediate to high yields of protein concentrate with low chlorogenic acid levels. Acid diffusion rates were temperature-dependent and 80°C was required to remove 90% of the chlorogenic acid from sunflower seeds. Important factors in assessing the relative merits of the various processes are the need for recovery of the solvent in the alcohol process, the relative ease in handling of products during acid diffusion of the seed, and the nitrogen solubility of the protein concentrates.

Sunflower flours are rich in protein and contain no known antinutritive factors, but are limited in food uses because of dark pigments (1). Chlorogenic acid is the principal phenolic constituent in sunflower seeds (2) that can be extracted from flours with organic solvents (3,4) or from seeds with acidic solutions (5). A continuous process for diffusion of chlorogenic acid required a solvent-to-seed ratio of 80:1 (6). In a commercial operation, the high volumes of extract liquor would create a serious problem of waste disposal or solvent recovery.

The objective of the present study was to decrease the solvent requirements for the extraction of chlorogenic acid from sunflower seeds and flours. The minimum solvent requirements were determined for three to five consecutive batch extractions with fresh solvent and for countercurrent extraction procedures in which the solvent was used in five to six stages before recycling. The extraction efficiencies of dilute acid, water, and ethanol were compared at room temperature and at 60°–80°C in the aqueous extractions. Solvent use, rate of chlorogenic acid extraction, and composition and quality of the protein concentrate were determined.

MATERIALS AND METHODS

Flour and Seed Preparation

Seeds of the Commander variety were used in the present study because the confectionery type is readily dehulled and provides a higher flour yield than the

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oilseed varieties. The seeds were dehulled in an experimental oat dehuller and the meats flaked to 0.01 in. thickness before oil extraction with Skellysolve F in a Soxhlet-type extractor. The defatted flakes were desolventized in a vacuum oven at 50°C and ground to 56 mesh in a Udy cyclone sample mill. For the seed diffusion experiments, the dehulled seeds were cracked into two or three pieces between corrugated rolls to facilitate testa removal and rapid diffusion (6). After diffusion, the seed pieces were dried on an open screen in a 50°C air oven and then flaked, defatted, desolventized, and ground as in flour preparation.

Extraction and Diffusion Procedures

Three to five extractions of 100-g samples of flour were conducted in 1000-ml plastic bottles, with continuous stirring at room temperature (24°C) or 80°C for 30 min each. The solvent-to-flour ratio was 5:1 (v/w) for each stage of the batch extraction procedure and 6:1 was used for the complete countercurrent process. During the aqueous extractions, protein losses were minimized by adjusting pH to the apparent isoelectric point of 4.5 (7) with 0.1 *N* HCl, or by first boiling the flour-water slurry for 15 min to denature the flour proteins. The 70% ethanol extraction process used the known insolubility of globulin proteins in 20–80% concentrations of organic solvents to control protein losses (8). The extracts were separated from the flour slurries by centrifugation for 15 min at 1500 × *g*, decanting the extract from the flour residue after each extraction.

Four to six diffusions of 200 g of seed pieces were conducted in 1000-ml plastic bottles in a shaking water bath at 60°, 65°, or 80°C for 30 min each. The solvent-to-seed ratios were 3:1 for both batch and countercurrent diffusion procedures. However, since the seeds contained over 50% oil, seed diffusions were actually conducted at an equivalent solvent-to-flour ratio of about 6:1. The solvent used for the diffusion experiments was dilute HCl at pH 4.5 and, after diffusion, the hot extracts were drained from the seeds through cheesecloth.

In the batch process, fresh solvent was used for each extraction; in the countercurrent procedure, fresh solvent was introduced in the final stage of a multistage system. For example, in a five-stage countercurrent procedure, the fresh solvent was applied to flour or seed that had been extracted four times previously, in order to remove the last traces of chlorogenic acid before the product was dried. This dilute extract would then be used to complete the fourth extraction and so on until the first stage in which the thick extract was applied to fresh flour or seed before being removed for chemical analysis.

After the flour extractions were completed, the wet protein concentrates were freeze-dried. If aqueous ethanol were used in the extraction, the organic solvent was evaporated at 50°C before freeze-drying. The wet seed pieces were dried in an air oven at 50°C before defatting with Skellysolve F.

Analytical Methods

The protein concentrates were analyzed for concentrate yield, protein content, nitrogen solubility at pH 7 and 9, and residual chlorogenic acid content and color of the concentrate at pH 9. Protein content was determined by the micro-Kjeldahl procedure (N × 6.25). The nitrogen solubility indexes (NSI's) were measured by the AACC procdure (9) at pH 7 and 9. The extracts from the batch and countercurrent extractions were analyzed for chlorogenic acid, protein, and total solids contents. The concentrations of chlorogenic acid in the extracts were

determined by the spectrophotometric procedure of Milic *et al.* (10) at 328 nm. Chlorogenic acid levels in the flour and protein concentrates were measured spectrophotometrically on 70% ethanol extracts obtained by stirring the ethanol-flour slurries (200:1) for 90 min. The adverse effects of residual chlorogenic acid on product color were determined by adjusting a dilute suspension (100:1 v/w) of protein concentrate to pH 9 and observing the color after standing for 60 min. The data reported in the tables are the average of duplicate determinations.

RESULTS AND DISCUSSION

Extraction Efficiency

Experiments with batch extractions demonstrated that three to five extractions of 30 min each were necessary to remove 90% or more of the 3.9% chlorogenic acid in sunflower flour. For example, three successive batch extractions of 100 g of flour with 500 ml of fresh 70% ethanol were required to remove a total of 3.5 g of chlorogenic acid (Table I). In the ethanol extraction, the 100 g of flour absorbed about 150 ml of solvent during the first extraction and after the third extraction, the 77 g of protein concentrate retained nearly 200 ml of solvent. The 1300 ml of combined extracts contained 23 g of soluble or suspended flour solids, including the 3.5 g of chlorogenic acid, which represented a solids load of 1.8%.

Three batch extractions of the flour with dilute acid removed only 3.0 g of chlorogenic acid and the process was extended to five stages to produce a low chlorogenic acid product (Table I). About 41.5 g of flour constituents was dissolved or suspended in 2300 ml of combined extract which also gave a dilute 1.8% solution.

To reduce the solids losses during aqueous extraction, dehulled sunflower seeds were diffused against dilute HCl at pH 4.5 for a similar series of 30-min batch extractions (Table I). The solvent-to-seed ratio was 3:1 v/w (*i.e.*, 600 ml to 200 g) in each extraction and four batch diffusions were required to obtain a low chlorogenic acid level in the protein concentrate. The solids content of the combined extract was only 1.3% on the basis of 28.5 g of solids in 2174 ml of extract.

During the batch extractions shown in Table I, the quantities of chlorogenic acid solubilized in each successive extraction were substantially decreased. It appeared logical to reuse the solvents in a systematic manner and improve the extraction efficiencies of the successive flour extractions and seed diffusions. A countercurrent extraction procedure was devised to utilize each batch of solvent until its chlorogenic acid concentration became too high for efficient removal of additional phenolic acid. Depending on the solvent and extraction conditions, it was found that five to six stages in the countercurrent system were required to remove about 90% of the chlorogenic acid from the flour or seed. To complete this number of countercurrent extractions with a single batch of fresh solvent, it was necessary to increase the solvent-to-flour ratio to 6:1, but the ratio for seed diffusions was satisfactory at 3:1. Raising the solvent-to-flour ratio above these levels improved the extraction efficiency only slightly, but served to markedly increase the final extract volume. For this reason, only the minimal ratios were employed in the present study, but the number of stages and other extraction conditions were varied to obtain the desired results.

It was necessary to use a five-stage countercurrent extraction with 70% ethanol to remove 3.6 g of chlorogenic acid from the flour (Table I). However, only 600 ml of fresh solvent was required for the process and this initial volume was reduced to 396 ml of extract after the fifth extraction. This extract contained 5.5% of solubles which could be recovered as a feed-grade by-product during solvent distillation and condensation for recycling in a commercial operation. About 210 ml of solvent could be recovered during the drying of the protein concentrate.

Problems were encountered with the countercurrent extraction of flour with

TABLE I
Distribution of Solvent Between the Flour and Extract, and Chlorogenic Acid in the Extract, at Each Stage of Batch and Countercurrent Extraction Process

Extraction Process and Component	Extractor and Stage				
	A	B	C	D	E
Batch extraction of flour with 70% ethanol, 24°C					
Before each extraction					
Fresh solvent, ml	500	500	500	...	...
Flour or concentrate, g	100	256	276	...	...
After each extraction					
Extract volume, ml	344	468	490	...	...
Chlorogenic acid in extract, g	2.2	0.9	0.4	...	...
Batch extraction of flour with dilute acid, 24°C					
Before each extraction					
Fresh solvent, ml	500	500	500	500	500
Flour or concentrate, g	100	244	256	260	268
After each extraction					
Extract volume, ml	340	488	495	495	495
Chlorogenic acid in extract, g	1.8	0.8	0.4	0.3	0.2
Batch diffusion of seed with dilute acid, 80°C					
Before each extraction					
Fresh solvent, ml	600	600	600	600	...
Dehulled seed, g	200	376	378	392	...
After each extraction					
Extract volume, ml	398	590	590	596	...
Chlorogenic acid in extract, g	1.9	0.9	0.4	0.2	...
Countercurrent extraction of flour with 70% ethanol, 24°C					
Before each extraction					
Fresh solvent, ml	...	...	...	...	600
Flour or concentrate, g	100	252	283	288	288
After each extraction					
Extract volume, ml	396	550	580	588	595
Chlorogenic acid in extract, g	3.6	2.3	1.6	0.8	0.3
Countercurrent diffusion of seed with dilute acid, 80°C					
Before each extraction					
Fresh solvent, ml	...	...	...	...	600
Dehulled seed, g	200	370	374	376	380
After each extraction					
Extract volume, ml	380	555	585	588	592
Chlorogenic acid in extract, g	3.6	2.7	1.7	0.9	0.3

aqueous solvents and results of these experiments are described in the following section.

Acid diffusion of sunflower seeds at 80°C by a five-stage countercurrent system was rapidly accomplished because the hot extract drained quickly from the seed pieces and could be immediately applied to the next batch of seed (Table I). The seed diffusion process was as efficient as alcohol extraction of the flour in chlorogenic acid removal. Solvent use in the countercurrent diffusion experiment was quantitatively similar to that of the alcohol process. About 200 ml of dilute HCl was absorbed by the seed and 380 ml of final extract contained 6.9% of solubles.

Batch Extraction

The yield, composition, and properties of protein concentrates and extracts from all batch extractions are presented in Table II. Three 30-min batch extractions of the flour at pH 4.5 reduced the chlorogenic acid from 3.9% in the flour to 1.0% in the protein concentrate, and the product developed a dark brown color above pH 7. Despite the inefficient extraction of the phenolic constituent, nearly 40% of the flour solids were solubilized by the acidic solvent and 23% of the proteins were lost in the extract. The acid treatment did improve the solubility properties of the protein concentrate at pH 7 and 9.

Water extraction of the denatured flour gave a protein concentrate with 0.7% of chlorogenic acid and white color characteristics, except above pH 8 (Table II). The yield of protein concentrate was greater than in the isoelectric extraction but the protein loss was 21% and the NSI's were very low.

The best solvent in these batch extractions was 70% ethanol which in three extractions removed 90% of the phenolic constituent and yet limited the solids losses to 23% and protein losses to only 5% (Table II). The protein concentrate was creamy-white in appearance over a broad range of pH and the proteins were only partially denatured.

When the acid extraction of the flour was increased to five stages, the chlorogenic acid content was reduced to 0.5% in the protein concentrate, and there was only a slight increase in protein loss as compared to the three-stage procedure (Table II). Acid extractions at 80°C were done at three to five stages but there was little gain in extraction efficiency and the NSI's were decreased substantially by the treatment.

Acid diffusion of the seed pieces in four batch extractions gave an intermediate yield of protein concentrate (71.4%) and protein losses were reduced to 17.3% (Table II). As shown previously, the semipermeable membranes in the cell walls of the seed were found to limit the losses of bound and high-molecular-weight compounds in seed diffusion experiments (5). Protein denaturation was quite extensive because of the high 80°C temperature used during acid diffusion.

Compared to acid extraction of the flour, the seed diffusion technique reduced solids and protein losses but not solvent volume. The use of 70% ethanol in flour extractions gave the highest yields of protein concentrate, limited nitrogen losses to essentially the nonprotein nitrogen in the flour, and reduced the required solvent-to-flour ratio to 15:1. Unfortunately, ethanol is the more expensive solvent and the extract contained only 1.8% of soluble constituents. The solvent recovery operation for such a dilute solution would be relatively expensive.

TABLE II

The Influence of Extraction Conditions on the Yield, Composition, and Properties of Protein Concentrates and Extracts Prepared by Batch and Countercurrent Procedures

Extraction (Ext.) and Diffusion (Diff.) Procedures	Number of Extractions	Total Solvent-to-Flour Ratio (v/w)	Extract Protein Loss %	Protein Concentrate		Nitrogen Solubility Index		Chlorogenic Acid in Product g/100 g	Color of Slurry pH 9
				Yield %	Protein Content %	pH 7	pH 9		
Control flour	...	...	...	...	59.2	26	90	3.9	Green
Batch extraction procedures									
Acid ext. of flour, 24°C	3	15	23.1	60.5	75.2	53	98	1.0	Brown
Water ext. of flour, 24°C	3	15	21.3	67.5	69.0	2	18	0.7	Yellow
Alcohol ext. of flour, 24°C	3	15	5.1	77.3	72.7	23	70	0.4	Creamy-white
Acid ext. of flour, 24°C	5	25	25.2	58.5	75.7	53	89	0.5	Yellow
Acid ext. of flour, 80°C	5	25	30.0	60.1	69.0	27	44	0.3	Creamy-white
Acid diff. of seed, 80°C	4	24	17.3	71.4	68.6	7	47	0.4	Creamy-white
Countercurrent extraction procedures									
Acid ext. of flour, 24°C	5	6	24.9	60.6	73.4	46	90	1.2	Green
Acid ext. of flour, 80°C	5	6	26.5	60.0	72.5	...	...	0.8	Light green
Acid ext. of flour, 24°C	6	6	26.1	57.2	76.5	57	94	0.5	Yellow
Alcohol ext. of flour, 24°C	5	6	2.8	78.0	73.8	18	76	0.4	Creamy-white
Acid diff. of seed, 80°C	5	6	12.1	73.8	70.5	9	41	0.4	Creamy-white
Acid diff. of seed, 65°C	6	6	17.0	69.6	70.6	13	69	0.8	Light green

Countercurrent Extraction

The five-stage countercurrent extraction of the flour with acid reduced the chlorogenic acid level to 1.2% in the protein concentrate (Table II). Raising the temperature for this treatment to 80° C failed to improve the color characteristics substantially but the six-stage extraction at room temperature provided a satisfactory lightly colored product. The total water use in each of these three acid extractions of the flour was only 6:1, and, because the yields of protein concentrate were only 57–61%, there was nearly 10% of dissolved and suspended solids in the extracts. As with the batch extractions, about 25% of the flour proteins were lost in the extract solubles but these would be recovered, after evaporative concentration, as an additive for feeds. The solubles would include chlorogenic acid, other phenolic compounds, sugars, sucrose, maltose, melibiose, raffinose (11), soluble proteins, nonprotein nitrogenous constituents, and minerals.

The five-stage countercurrent extraction of the flour with 70% ethanol gave a high yield of protein concentrate (78%) which contained only 0.4% of chlorogenic acid (Table II). The protein content of the product was high (73.8%) because nitrogen losses in the extract solubles were very low (2.8%). Protein solubility at pH 7 was relatively low but increased to 76% at pH 9.

Acid diffusion of sunflower seeds at 80° C by the five-stage countercurrent system was also efficient in the removal of chlorogenic acid; product yield was high (73.8%), and nitrogen losses in the extract solubles were limited to 12.1% by the seed membranes (Table II). Protein solubility was, however, substantially lower than the alcohol-washed flours. Diffusion extraction of seed was also done at 60° C and the protein concentrate contained 1.3% of chlorogenic acid. A six-stage countercurrent diffusion experiment at 65° C reduced the chlorogenic acid level to 0.8% in the final product, and protein solubility was improved to almost the same level as the alcohol-extracted concentrate (Table II). In this six-stage procedure, the product yield was lower and protein losses higher than in the corresponding five-stage diffusion at 80° C.

General Discussion

Although not dealt with specifically in the present study, the seed diffusions were accomplished much more readily than the flour extractions. For example, the seed and solvent were more easily agitated than the viscous flour slurries. Seed and extract were rapidly separated between extractions by drainage through cheesecloth, whereas centrifugation was required to separate the extracted flour and the viscous extract. Similarly, the seeds were rapidly dried on an open screen in an air-oven while freeze-drying was used for the extracted flours to avoid adverse physical and color characteristics in the dried product. While the handling problems were somewhat reduced in the alcohol extraction system, the solvent is more expensive and the mandatory solvent recovery system would add greatly to the cost of ethanol extractions. Therefore, the seed diffusion technique offered a useful alternative to the well-known flour extraction procedures for the removal of soluble constituents and the preparation of protein concentrates. The countercurrent diffusion of seeds could be readily adapted to a continuous countercurrent flow system, while flours would be most efficiently processed in a stationery batch system since vacuum, pressure, or centrifugation would be required to separate wheys from the flour-solvent slurry.

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Literature Cited

1. SOSULSKI, F. W., and BAKAL, A. Isolated proteins from rapeseed, flax and sunflower meals. Can. Inst. Food Technol. J. 2: 28 (1969).
2. SABIR, M. A., SOSULSKI, F. W., and KERNAN, J. A. Phenolic constituents in sunflower flour. J. Agr. Food Chem. 22: 572 (1974).
3. GHEYASUDDIN, S., CATER, C. M., and MATTIL, K. F. Preparation of a colorless sunflower protein isolate. Food Technol. 24: 242 (1970).
4. POMENTA, J. V., and BURNS, E. E. Factors affecting chlorogenic, quinic and caffeic acid levels in sunflower kernels. J. Food Sci. 36: 490 (1971).
5. SOSULSKI, F. W., McCLEARY, C. W., and SOLIMAN, F. S. Diffusion extraction of chlorogenic acid from sunflower kernels. J. Food Sci. 37: 253 (1972).
6. SOSULSKI, F. W., SABIR, M. A., and FLEMING, S. E. Continuous diffusion of chlorogenic acid from sunflower kernels. J. Food Sci. 38: 468 (1973).
7. KILARA, A., HUMBERT, E. S., and SOSULSKI, F. W. Nitrogen extractability and moisture absorption characteristics of sunflower seed products. J. Food Sci. 37: 771 (1972).
8. CIRCLE, S. J., and SMITH, A. K. Processing soybeans, protein concentrates, and protein isolates. In: Soybeans: Chemistry and technology, ed. by A. K. Smith and S. J. Circle, p.294. AVI Pub. Co.: Westport, Conn. (1972).
9. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC (7th ed.). The Association: St. Paul, Minn. (1962).
10. MILIC, B., STOJANOVIC, S., VUCUREVIC, N., and TURCIC, M. Chlorogenic and quinic acids in sunflower meal. J. Sci. Food Agr. 19: 108 (1968).
11. SABIR, M. A., SOSULSKI, F. W., and HAMON, N. W. Sunflower carbohydrates. J. Agr. Food Chem. 23: 16 (1975).

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