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Preparation of Colorless Sunflower Protein Products: Effect of Processing on Physicochemical and Nutritional Properties

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ABSTRACT

A comparison was made of the different technological treatments for the preparation of colorless sunflower protein products from the viewpoint of the effect of processing conditions on the extraction yield of nitrogen and lipid, chemical, physicochemical and nutritional properties of the processed products. The technological treatments comprised soaking dehulled seeds in dilute citric acid or sodium bisulfite solution and washing the defatted meal with the respective solution. The defatting process was carried out with hexane or azeotrope (hexane/ethanol). Nitrogen and lipid recovery was slightly greater for hexane defatted products than for azeotrope defatted products. About 21.4% of the phenolic compounds of the sunflower seeds were bound to the proteins of the seeds before processing and therefore could not be eliminated by the aqueous extraction. Aqueous extraction of phenolic compounds was limited for full fat seed. The free phenolic compounds were very stable in acid medium but sensitive to oxidation in alkaline medium and had no significant effect on in vitro enzymatic proteolysis and growth inhibition of rats. Lysine and the bound phenolic compounds were the critical factors responsible for inhibition of enzymatic proteolysis and reduced growth of rats. The diet containing whole seed meal presented a low protein efficiency ratio (PER) value. Citric acid, a chelating agent, proved to be an antioxidant as effective as sodium bisulfite; the products obtained by citric acid treatment had a visually whiter color than those processed by sodium bisulfite.

INTRODUCTION

The potential uses of sunflower proteins are limited by the presence of phenolic substances, particularly chlorogenic acid (1). The isolation of protein and removal of chlorogenic acid from sunflower meal have been investigated by numerous scientists. Various methods have been devised. These include extraction of the meal with aqueous ethanol (2, 3), aqueous methanol (4), acidic butanol (5) and NaCl solution (6). Gheyasunddin et al. (7) produced a colorless sunflower protein isolate by treating the soluble protein with sodium sulfite and washing the precipitated protein with 50% isopropanol, and Sosulski et al. (8) produced stable white concentrate by hot aqueous diffusion of cracked sunflower kernels.

However, these processes were hardly feasible for industrial use. The high volumes of extract liquor from aqueous solvent processes would create a serious problem of water disposal or solvent recovery for commercial application. The diffusion process was very temperature-dependent. Due to the long extraction periods, the large volumes of water and high temperature, the operational costs of the batch diffusion methods would be prohibitive. In addition, these processes caused a greater loss of proteins and lipids, and alcohol may denature a protein by destroying its native configuration (9, 10). An excessive protein denaturation is not desirable for some food applications (11). In view of extending the utilization of sunflower proteins in human foods, a simple economical process has to be developed; the operational costs and the feasibility for industrial use and commercial application should be taken into account.

Colors and flavors of sunflower protein products are associated with the presence of hulls, polyphenolic compounds and low molecular weight carbohydrates. The hydrogen bonding between the hydroxyl groups of phenolic compounds and the peptide bonds in proteins is known to be unusually strong and the equilibrium in aqueous solution strongly favored the formation of complexes (12). Therefore, the complete extraction of chlorogenic acids from sunflower flour with polar organic, aqueous, or aqueous/organic solvents would be difficult to achieve. The extraction with NaCl solution could induce discoloration of the proteins, since minerals can accelerate oxidation of phenolic compounds. Chlorogenic acid is readily oxidized to quinone, both nonenzymatically by oxygen at alkaline pH and enzymatically in the vicinity of neutral pH by polyphenol oxidase (13), therefore during the extraction procedure of chlorogenic acid, the use of a reducing agent is necessary.

A systematic and comprehensive study of conditions af-

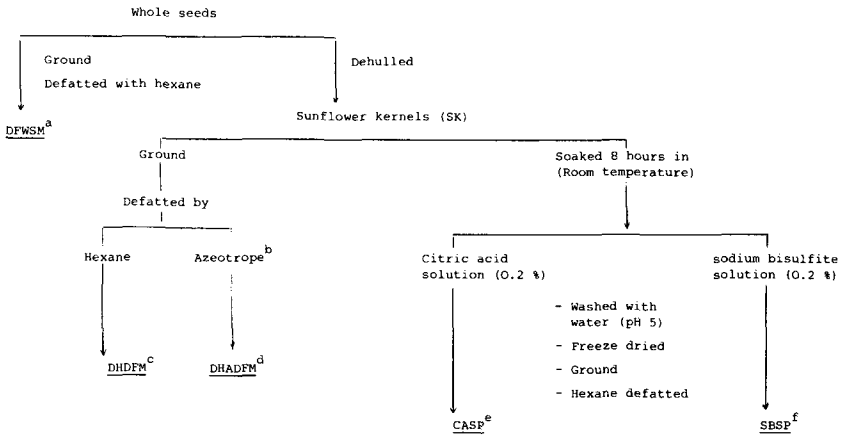

FIG. 1. Preparation of protein products from sunflower seeds. (a) Defatted whole seed meal; (b) azeotrope (hexane/95% ethanol, 8/2, v/v); (c) dehulled and hexane defatted meal; (d) dehulled and azeotrope defatted meal; (e) citric acid soaked product; (f) sodium bisulfite soaked product.

fecting the extraction of sunflower proteins is important from the industrial as well as the scientific standpoint for wide utilization of sunflower proteins in human foods in the future. Improved procedures for preparation of sunflower protein products would require further investigation.

The object of this study was to evaluate the different technological treatments from the point of view of the effect of processing on the chemical composition, especially phenolic compounds and composition of dietary fiber of the processed products. We determined also the extraction yield of proteins and lipids, chemical, physicochemical and nutritional properties of processed products for the purposes of improving the utilization of sunflower proteins in human foods.

MATERIALS AND METHODS

Preparation of Protein Products

The various dehulled, defatted sunflower meal fractions used in this study were prepared from commercial, untreated sunflower seeds (Société Huilerie J. Bulhon, France) according to the procedures described in Figures 1 and 2. This commercial high oil variety contains ca. 27% hulls, 2% kernel membrane, 19% protein, 47% oil and 2.5% ash. The whole sunflower seeds were divided into two portions. One portion was ground directly into powder with a grinder "vertec" (Verre et Technique, Arceuil, France), then defatted with hexane, and the other portion was dehulled manually. The dehulled sunflower kernels (SK) were divided into two portions again, one portion was ground directly into powder and then defatted with hexane (DHDFM) and azeotrope (DHADFM), respectively. The other portion was separated into two groups and soaked in citric acid solution (0.2%) and sodium bisulfite solution (0.2%), respectively, for 8 hr at room temperature. The soaked beans were then washed with water, freeze-dried, ground and defatted by hexane (Fig. 1). The dehulled sunflower seeds dehulled manually in our laboratory were divided into two portions. One portion was soaked in citric acid solution (0.2%) at 100 C for 20 min (seeds solution, 1:10) and the other portion in sodium bisulfite solution in same condition to inactivate polyphenol oxidases and to remove soluble phenolic substances, then washed with deionized water. After elimination of residual hulls by floating, the treated seeds were dried by warm air at 50–60 C using a preheated autoclave system and finally ground to powder. The powder was redivided into two portions. One portion was defatted with hexane and the other portion with an azeotrope (hexane/ethanol 95%, 8:2 v/v).

The defatted meal was suspended again in sodium bisulfite solution and citric acid solution, respectively (meal solution, 1:10, w/v) at ambient temperature for 1 hr, for the purpose of further elimination of phenolics, then washed, rinsed in deionized water and freeze-dried prior to functional and nutritional analysis (Fig. 2).

For brevity, the samples were designated as follows: SK = sunflower seed kernels, DFWSM = defatted whole seed meal, DHDFM = dehulled and hexane defatted meal, DHADFM = dehulled and azeotrope defatted meal, CASP = citric acid soaked product, SBSP = sodium bisulfite soaked product, SBHDP = sodium bisulfite treated and hexane defatted product, SBADP = sodium bisulfite treated and azeotrope defatted product, HDP = citric acid treated and hexane defatted product, and ADP = citric acid treated and azeotrope defatted product.

Chemical Analyses

Nitrogen was measured by the micro-Kjeldahl procedure (14) and dietary fiber was estimated by Southgate procedure (15). Hull and kernel membrane were separated manually in our laboratory. All the samples for dietary fiber determination were freeze-dried, sugar- and fat-free. These sugar- and fat-free samples were carried through the fractionation. The hydrolysates obtained from different fractionations were passed through a column of a mix of ion exchange resin AG 501-X8 (D) (Bio-Rad, Richmond, CA) and activated carbon (DARCO) (Atlas Chemical Industries, Wilmington, DE) for desalting and decolorizing before spectrophotometer measurement. Lipid content was determined by soxhlet extraction with a mixture of petroleum ether and diethyl ether (1:1, v/v). Sugars were extracted with 80% alcohol (16) and measured by the anthrone method (17). The colors of processed products were noted visually.

Nitrogen Solubility Determination

The sample was dispersed in water up to a final concentration of 4% dry weight and solid NaCl or $CaCl_2$ was dissolved in the mixture with the concentration of 0.25, 0.5, 0.75 . . . 6M for NaCl and 0.025, 0.05, 0.01 . . . 2M for $CaCl_2$. The pH was adjusted to 2, 3, 4 . . . 10 by careful addition of 1 N NaOH or HCl. The suspension was shaken

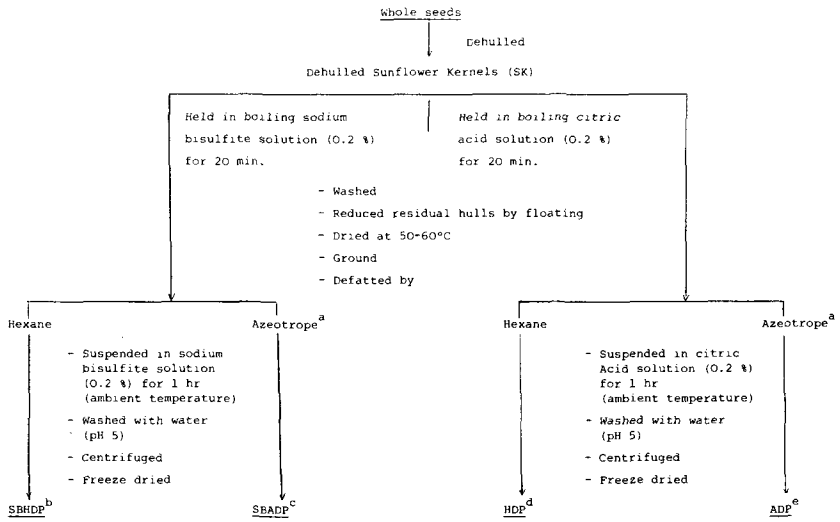

FIG. 2. Preparation of protein products from sunflower seeds. (a) Azeotrope (hexane/95% ethanol, 8/2, v/v), (b) sodium bisulfite treated and hexane defatted product; (c) sodium bisulfite treated and azeotrope defatted product; (d) citric acid treated and hexane defatted product; (e) citric acid treated and azeotrope defatted product.

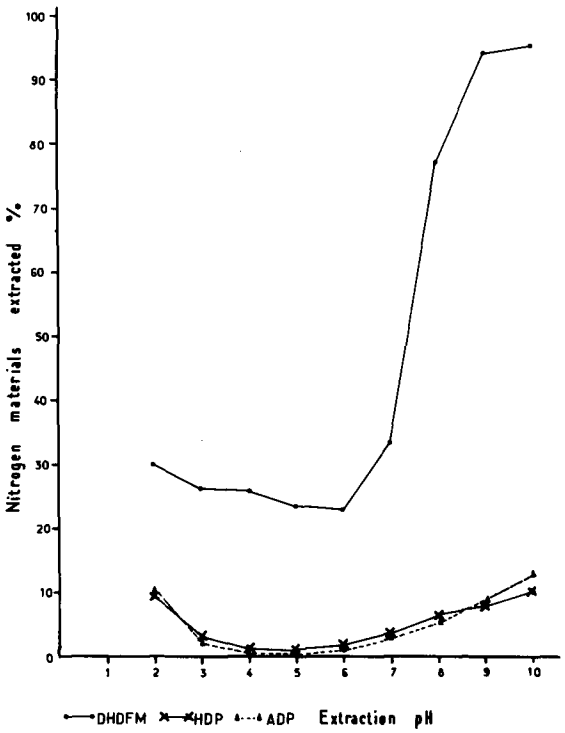

FIG. 3. Nitrogen solubility of hexane defatted sunflower meal and processed products as a function of pH. (Temperature = 25 C; concentration of dry material = 4%.)

for 2 hr at room temperature (20 C), then centrifuged at 4,000 rpm for 25 min. The clear supernatant was filtered through a Whatman filter paper (no. 54). Total nitrogen was determined by micro-Kjeldahl analysis on filtered supernatant.

Determination of Phenolic Compounds

Phenolic compounds were measured according to the method of Monties and Rambourg (18). Three to five hundred mg of finely ground sample mixed thoroughly with 25 mL acid-washed sand was transferred into a column. The sample was eluted with the following solvents: petroleum ether containing 2% methanol (extraction of chlorophyll pigments, carotenoids and lipids); diethyl ether (final extraction of liposoluble pigments); methanol and distilled water (extraction of polyphenolic compounds).

The eluted methanol and distilled water extracts were separately collected in a brown graduated flask containing a drop of 6 N hydrochloric acid. Content of polyphenolic compounds was measured by absorbancy at 330 nm. To analyze for protein-bound phenolic substances, the samples were defatted and cleared of soluble phenolic compounds by first washing with methanol and water, and then treating with a mixture of acetyl bromide and glacial acetic acid (1:3, v/v). This treatment dissolved cell walls and released the bound phenolic compounds (18).

Amino Acid Analysis

Amino acid analysis was carried out in duplicate according to the method of Moore et al. (19), using a Biotronik LC-6000 E autoanalyzer (Wissenschaftliche Geräte GMbM, West Germany). Cystine and methionine were determined by the procedure of Schram et al. (20) after oxidation to cysteic acid and methionine sulfone, respectively.

In vitro Digestibility

Digestibility measurements with trypsin-pepsin and pepsin-pancreatin were carried out, with some modification, according to Saunders et al. (21). Enzymatic hydrolysis was terminated by the addition of trichloroacetic acid (0.8 M final concentration). The measure of the amount of nitrogen soluble in 0.8 M TCA (micro-Kjeldahl), referred to as nonprotein nitrogen (NPN), was used to determine the extent of enzyme proteolysis. The calculation is as follows:

$$\text{Enzymatic digestion (\%)} = \frac{\text{N (NPN) released by enzyme}}{\text{N in sample}} \times 100$$

Trypsin (SERVA from bovine pancreas, 40 μ/mg, 2 x crystallized, reference 37260) was suspended in tris-buffer (tris-hydroxymethyl aminomethane, 0.05 M, pH 8.2). Pepsin (SERVA pepsin porcine, 15 millianson units/mg, 2 x crystallized, reference 31820) digestion was carried out in 0.06 M HCl, pH 1.8. Pancreatin (SIGMA from porcine pan-

TABLE I

Proximate Analyses and Nitrogen and Lipid Recoveries in Sunflower Meal (dry weight basis)

Sample[a]	Protein (%)	Lipid (%)	Ash (%)	Ethanol soluble sugars (%)	Starch (%)	Solid recovery (%)	Nitrogen recovery (%)	Lipid recovery (%)
Hull	2.80	1.20	2.50	0.20				
SK[b]	22.80	57	2.80	2.20				
DFWSM[c]	33	1–3	5.87	1.26				
DHDFM[d]	53	1–3	7.85	2	0.42			
HDP[e]	63	1–3	2.22	0.20	0.25	82	97	93
ADP[f]	65	2–4	2.02	0.10	0.18	77	94	92
SBHDP[g]	64	1–2	7.50	0.15	0.16	79	95	91
SBADP[h]	66	1–3	7.40	0.06	0.12	75	93	90

[a]See text, Figures 1 and 2 for details.
[b]Sunflower seed kernels.
[c]Hexane defatted whole seed meal.
[d]Dehulled hexane defatted sunflower meal.
[e]Hexane defatted product.
[f]Azeotrope defatted product.
[g]Sodium bisulfite treated and hexane defatted product.
[h]Sodium bisulfite treated and azeotrope mixture defatted product.

creas, grade II reference p-1500) digestion was carried out in 0.2 M phosphate buffer, pH 8, containing 0.005 M sodium azide.

Rat Bioassays

Samples for protein efficiency ratio (PER) determinations were freeze-dried and ground into a powder (100 mesh). Weanling male rats weighing 55–65 g (Wistar strain) at 28 days of age (10 rats/diet) were fed ad libitum during 28 days with a diet containing 10% protein (dry basis). PER values were calculated for both experimental and standard diets (AOAC) (14): PER values of meal fractions supplemented with 1% (+) lysine (lysine/protein; dry basis) were also determined.

RESULTS AND DISCUSSION

Aqueous Extraction Procedure

The technology involved in the aqueous procedure used (Fig. 3) is simple, inexpensive, and can be adapted to other types of high protein seeds such as rapeseed, cottonseed, soybean, safflower seeds, etc. This process had a high yield of nitrogen and lipid recovery (>90%) and eliminated almost all the ethanol-soluble sugars, residual seed hulls, more than 84% of free phenolic compounds and over 70% of the ash of sunflower kernels. The processed products are white in color, they contain more than 60% proteins and ca. 25% total dietary fiber; their PER values can equal that of casein when 1% lysine is added.

Composition of Products

Proximate analyses of the various protein products are summarized in Table I. The samples defatted by azeotrope mixture (ADP, SBADP) contain slightly more protein than those (HDP, SBHDP) defatted by hexane. Ash content for HDP (2.02%) and ADP (2.30%) was much less than that for DHDFM (7.85%), SBHDP (7.5%) and SBADP (7.4%). Therefore, boiling citric acid solution (0.2%) was effective in reducing ash content, whereas similar treatment with sodium bisulfite solution did not reduce ash content in the processed products. Citric acid treated products had a visually whiter color than those treated by sodium bisulfite. High ash content could be one of the factors inducing discoloration of the products, since minerals can accelerate oxidation of phenolic compounds. Boiling citric acid and sodium bisulfite treatment (Fig. 2) were equally effective in removing most of the soluble sugars.

Extraction Yield

The results given in Table I refer to laboratory assay runs with a batch size of 2 kg of dehulled seeds. The extraction process described in Figure 2 eliminated almost all the ethanol-soluble sugars, whereas loss of protein and lipids was negligible. Loss of 10–12% solids as a result of 0.2% citric acid and sodium bisulfite treatment (20 min at 100 C), consisted of soluble phenolic substances, ethanol-soluble sugars, hull residues, minerals and a little trace of oil. After the defatting stage, the washing procedure caused a 8–10% further loss of dry material including soluble phenolic compounds, ethanol-soluble sugars and a small quantity of low molecular weight carbohydrates and proteins. This loss is slightly greater for ADP and SBADP than for HDP and SBHDP. Probably, the ethanol in the azeotrope mixture causes some protein denaturation to occur and results in the greater release of soluble phenolic compounds, sugars, minerals and other nonprotein constituents bound to the crude proteins. This process had a high yield of nitrogen and lipid recovery because protein loss was minimized by adjusting pH to the apparent isoelectric point of 5 and by first boiling the dehulled seeds for 20 min to insolubilize the proteins during the aqueous extraction procedure.

Composition of Dietary Fiber

Dietary fiber values are given in Table II. The ash values were calculated separately from lignin and are given in Table I. Sunflower hulls have a high lignin content compared to the kernel membrane. Dietary fiber content and composition of the fiber in the products DHDFM, DHP and ADP are very similar. The lignin content of these products is very low, indicating that hulls were effectively removed during processing; on the other hand, the kernel membrane is not eliminated from the seeds during the extraction process. The kernel membrane contains a large amount of hemicelluloses (29%) which are important in human physiology due to water-holding capacity, digestibility and capacity to bind ions. The kernel membranes can

TABLE II

Composition[a] of Dietary Fiber

Sample[b]	Total dietary fiber (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)
Hull	72.55	20.62	22.76	29.17
Kernel membrane	46.03	29.04	12.86	4.13
DHDFM[c]	19.07	11.45	4.45	3.17
HDP[d]	23.40	12.15	5.38	5.87
ADP[e]	23.36	12.89	6.14	4.33

[a]Dry basis.
[b]See text, Figures 1 and 2 for details.
[c]Dehulled and hexane defatted sunflower meal.
[d]Hexane defatted product.
[e]Azeotrope defatted product.

be a potential fiber additive because of their rich hemicellulose content.

Phenolic Compounds Content

We have tried different treatments (Figs. 1 and 2) in an attempt to eliminate phenolic compounds at various stages of the process. Table III gives the content of phenolic compounds of different processed products. Seed hulls contain small amounts of free phenolics (0.14%) but high amounts of bound phenolic compounds (4.41%). Defatted whole seed meal (DFWSM) contains 4.61% total phenolic compounds, of which 45.35% are soluble and 54.65% are bound. These data indicate that hulls account for most of the bound phenolics in DFWSM since it contains ca. 47% seed hulls. We found 2.99% phenolic substances in hexane defatted dehulled meal (DHDFM) of which 78.6% was soluble and 21.4% was protein-bound, this result reveals that ca. 21.40% phenolic compounds of the dehulled sunflower kernels were not soluble but were bound to proteins before aqueous extraction. Some bound phenolic compounds of seed hulls and defatted whole seed meal could come from the degradation of lignin during the polyphenol analysis using acetyl bromide and glacial acetic acid treatment, as seed hulls and DFWSM have a high lignin content.

In hexane/ethanol azeotrope defatted dehulled meal (DHADFM), we found 2.45% phenolics, of which 67.35% were soluble and 32.65% were protein-bound. The azeotrope defatting process eliminated a small part of soluble phenolics. Only ca. 40% free phenolics were extracted from the nondefatted whole seeds (CASP, SBSP), while more than 84% of the free phenolics were extracted from the defatted meal (SBHDP, SBADP, HDP and ADP) (Table III). These results suggest that aqueous extraction of phenolic compounds was limited for full fat seeds, the oil and the phospholipids of which form a hydrophobic region that protects the phenolic compounds from being solubilized. All the aqueous extracted samples contain more bound phenolics than defatted dehulled meal (DHDFM). It would appear that the bound phenolics can not be eliminated by aqueous extraction and the soluble phenolics are very sensitive to oxidation, so it is indispensable to use an antioxidant during extraction procedure. In our experiments, citric acid, a chelating agent, proved to be an antioxidant as effective as sodium bisulfite; the products obtained by citric acid treatment (HDP, ADP) had a visually whiter color than those (SBHDP, SBADP) processed by sodium bisulfite.

TABLE III

Phenolic Content of Sunflower Protein Products (dry weight basis)

Sample[a]	Phenolic content (%)	Phenolic compounds (%)[b]	
		Free	Bound
Hull	4.55	3.08	96.92
DFWSM[c]	4.61	45.35	54.65
DHDFM	2.99	78.60	21.40
DHADFM	2.45	67.35	32.65
CASP	2.2	67.73	32.27
SBSP	1.99	64.82	35.18
SBHDP	1.01	14.85	85.15
SBADP	0.92	14.13	85.87
HDP	1.23	24.39	75.61
ADP	1.26	29.37	70.63

[a]See Figures 1 and 2 for details.
[b]Percentage of total phenolic content in sample.
[c]Hexane defatted whole seed meal.

Nitrogen Solubility of Hexane Defatted Sunflower Meal and Processed Products as a Function of pH

The pH of the defatted sunflower meal (DHDFM) dispersions of 4% dry material (w/v) was varied from 2 to 10. The solubility curve of sunflower meal protein (Fig. 3) showed a broad minimum at pH 5–6, corresponding to the isoelectric region, with less than 24% of the nitrogen being soluble. Addition of acid increased nitrogen solubility from ca. 24% at pH 6 to 30% at pH 2. Extraction of nitrogenous material increases rapidly above pH 7 to a maximum value of ca. 94.26% at pH 9. Low protein solubility is a shortcoming for use of sunflower meal in slightly acidic beverages. Our extraction process (Fig. 2) insolubilizes sunflower proteins, causing the processed products (HDP, ADP) to have very low levels of solubility. Maximum extracted nitrogen for HDP and ADP was only 10 and 12.7%, respectively, at pH 10 (Fig. 3).

Nitrogen Solubility of Hexane Defatted Sunflower Meal and Processed Products in NaCl and $CaCl_2$ Solution of Different Concentration

The majority of the sunflower seed proteins are globulins, insoluble at their isoelectric point (pH 5–6). Poor water solubility of sunflower proteins close to neutral pH (6.5) was confirmed by the extractability curve (Fig. 3), but the nitrogen solubility can be increased in dilute sodium and calcium chloride solutions. Nitrogen solubility increases, at constant pH, as the salt concentration is increased (salting-in). It is also well known that salting-in of proteins

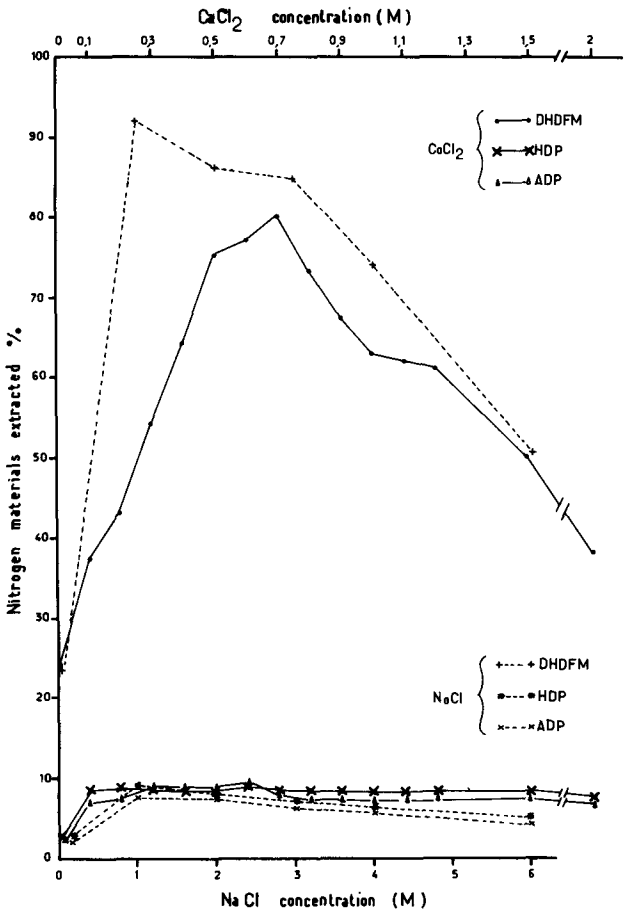

FIG. 4. Nitrogen solubility of hexane defatted sunflower meal and processed products as a function of NaCl and $CaCl_2$ concentration (M). (Temperature = 25 C; concentration of dry material = 4%; pH = 6.5.)

may be followed by salting-out when the ionic strength is increased sufficiently. NaCl and $CaCl_2$ play an important role in the nitrogen solubility of sunflower globulins. Figure 4 illustrates the solubility behavior of defatted sunflower meal (DHDFM) as a function of NaCl concentration for a 4% protein solution at pH 6.5, close to the natural pH of the seeds. Nitrogen solubility of sunflower meal increased as the addition of NaCl increased up to 1 M concentration. Maximum solubility (92–88%) was obtained at 1–1.5 M NaCl. This could be the point at which the salting-out stage starts, since nitrogen solubility decreased rapidly as NaCl concentration was increased to 3 M. The effect of $CaCl_2$ on the solubility of sunflower proteins did not follow the same pattern. Maximum nitrogen solubility (80%) occurred at 0.7 M $CaCl_2$. More than 70% nitrogen was extracted from sunflower meal with 0.5–0.8 M $CaCl_2$ at the natural pH of the seeds (pH 6.5). The nitrogen solubility of sunflower meal was 63.38% at 1 M $CaCl_2$, 38.19% at 2 M $CaCl_2$ (Fig. 4). The results obtained show that sunflower proteins are highly soluble in low concentration of NaCl and $CaCl_2$ solution, ranging from 1 to 2 M for NaCl, 0.5–0.8 M for $CaCl_2$.

Although the solubility of sunflower proteins increased in diluted sodium and calcium chloride solution, there was little increase for processed products (HDP and ADP) as their proteins were insolubilized by the thermal treatment. Maximum extracted nitrogen for HDP and ADP was only 9.13 and 8.19%, respectively, at a NaCl concentration of 0.75 M (Fig. 4). A broad maximum solubility (ca. 8.5%) was obtained with 0.3–1.5 N $CaCl_2$ at pH 6.5 for HDP and ADP (Fig. 4).

TABLE IV

Amino Acid Composition of Meal Prepared from Defatted Sunflower Kernels (g/16 g N)

Amino acid	Sample[a] DHDFM	HDP	ADP	FAO[b]
Lysine	3.5	3.4	3.2	5.4
Histidine	2.4	2.4	2.3	
Arginine	8.3	8.9	8.5	
Aspartic acid	8.9	8.9	9.1	
Threonine	3.3	3.6	3.5	4.0
Serine	4.1	4.4	4.4	
Glutamic acid	19.5	19.8	21.2	
Proline	4.5	4.3	4.0	
Glycine	5.0	5.1	4.9	
Alanine	4.2	4.2	4.2	
Methionine	2.3	2.3	2.2	3.5
Cystine	1.4	1.3	1.3	
Valine	4.8	4.8	4.6	5.0
Isoleucine	4.1	4.1	3.8	4.0
Leucine	6.2	6.3	6.1	7.8
Tyrosine	2.6	2.7	2.6	6.1
Phenylalanine	4.8	4.8	4.7	
Ammonia	1.8	1.3	1.2	
Total	91.7	92.6	91.8	

[a]See text, Figures 1 and 2 for details.

[b]Food and agriculture organization reference protein (FAO/WHO 1973).

Amino Acid Composition

Amino acid content of the various meal products, compared with that of raw dehulled and defatted sunflower meal (DHDFM) is given in Table IV. No significant differences were observed in essential amino acid content, except for lysine which was slightly reduced for HDP and ADP (3% and 9%, respectively). This loss was probably due to the aqueous extraction process with lysine passing into serum with the low molecular weight proteins and phenolic compounds. Also lysine is readily affected by oxidation of chlorogenic acid. Thus more attention should be paid to effects of the polyphenols and polyphenol oxidases present in the sunflower protein products.

In vitro Digestibility

Table V shows the variation in digestibility by pepsin-trypsin and pepsin-pancreatin. For the pepsin-trypsin proteolysis, the major part of proteins was hydrolyzed for all the samples in acid medium by pepsin (48 hr), less than 28% protein was left for a further 16-hr-long trypsin hydrolysis, thus all the samples present a high-pepsin-trypsin proteolysis rate (>89%). As for pepsin-pancreatin digestion, only ca. 50% protein was digested by pepsin after a 3-hr-long proteolysis. Another 50% protein was left in phosphate buffer pH 8 for further 24-hr-long pancreatin hydrolysis. In this alkaline medium, it was surmised that the most soluble phenolics were oxidized to form protein-bound complexes which are resistant to pancreatin proteolysis. DHDFM had the lowest bound phenolic content (21.4%) but the highest free phenolic content (78.6%) among the studied samples. DHDFM presented the highest pepsin proteolysis rate, 79.1% after 48 hr digestion and 58.6% after 3 hr proteolysis but the lowest pancreatin proteolysis rate, namely 15.2% out of 41.4% protein content after 24 hr. These results prove that protein-bound phenolic compounds inhibited protease digestion. Free phenolic compounds have no effect on enzymatic proteolysis and are

TABLE V

Pepsin-Trypsin and Pepsin-Pancreatin Digestibility of Sunflower Products

	Protein digestibility (%)[a]					
	Pepsin-trypsin proteolysis			Pepsin-pancreatin proteolysis		
Sample[b]	Pepsin (48 hr) proteolysis	Trypsin (16 hr) proteolysis	Total (pepsin-trypsin)	Pepsin (3 hr) proteolysis	Pancreatin (24 hr) proteolysis	Total (pepsin-pancreatin)
DFWSM[c]	72.6	17.1	89.7	51.5	20.4	71.9
DHDFM	79.1	12.4	91.5	58.6	15.2	73.8
DHP	78.2	13.6	91.8	46.2	30.0	76.2
ADP	78.7	17.9	96.6	48.8	32.8	81.6

[a]Protein digestibility (%) = $\frac{\text{N released by enzyme}}{\text{N in sample}} \times 100$.

[b]See text, Figures 1 and 2 for details.

[c]Hexane defatted whole seed meal.

TABLE VI

Nutritive Value of Sunflower Protein Products

	PER[b] (± sd)	
Sample[a]	Diet	Diet + 1% L(+) lysine (lysine/protein, w/w)
Caseine	2.50 ± 0.10	
DFWSM[c]	1.80 ± 0.08	2.09 ± 0.06
DHDFM[d]	1.90 ± 0.08	2.39 ± 0.10
HDP[e]	1.83 ± 0.06	2.15 ± 0.08
ADP[f]	1.63 ± 0.11	2.28 ± 0.09

[a]See text, Figures 1 and 2 for details.

[b]Protein efficiency ratio corrected on a basis of PER = 2.5 for casein.

[c]Hexane defatted whole seed meal.

[d]Dehulled and hexane defatted meal.

[e]Hexane defatted product.

[f]Azeotrope defatted product.

very stable in acid medium (0.1 N HCl). This could explain why DHDFM with high free phenolic content exhibited a high pepsin proteolysis rate. On the contrary, free phenolic compounds are very sensitive to oxidation in alkaline medium.

If the combined effect of pepsin, trypsin and pancreatin is taken into account, it would appear that the processed products HDP and ADP have a high proteolysis rate because of their low content of free and bound phenolic compounds.

Neucere et al. (22) described that for simple polyphenols interacting with a specific protein (arachin) in vitro, digestibility was either enhanced or impaired slightly, depending on the concentration of a simply polyphenol. If their results are taken into account, we could conclude that the effect of the presence of plant phenols and polyphenols on the nutritional status of proteins depends on the formation of phenol-protein complexes and the native properties of proteins and phenolic substances.

Rat Bioassay

The nutritional values of meal and protein products prepared from defatted sunflower kernels, as measured by PER, are given in Table VI. Dehulled and defatted meal (DHDFM), had the highest PER value (1.90), whereas ADP had the lowest PER value (1.63). DHDFM had the highest free phenolic compound content (78.6%) of total phenolic content (2.99%) but the lowest concentration of protein-bound phenolic compounds of all assayed samples. It would appear that free phenolic compounds do not inhibit the growth of rats. Supplementary lysine (1%) increased PER values of all diets. The greatest supplementary effect was obtained with a diet containing ADP. These results indicate that lysine was a limiting amino acid. Lysine content of all the test diets was below the rats' nutritional requirements. For the processed products (HDP, ADP), their lysine content and PER values were slightly lower than that for defatted sunflower meal (DHDFM). The PER value of HDP was somewhat higher than that for ADP but an inverse result was found with supplementary lysine (1%). This indicates that the protein-bound phenolic content also had a growth inhibitory effect, because the protein-bound phenolic content of HDP (31.10%) was somewhat higher than that of ADP (29.7%) and of DHDFM (21.4%). The diet of DFWSM contains ca. 14% hull, of which 4.2% in crude lignin, 3.18% cellulose and 2.89% hemicellulose; this diet had a lower PER value compared with DHDFM diet. DFWSM diet gave the lowest PER value of all samples supplemented with lysine. The low PER value of DFWSM supplemented with lysine may be due to the high content of nonpurified plant fibers which perturb the intestinal absorption of minerals and available energy. Even though DFWSM contains a rather high bound phenolic content, in vitro digestibility remains high.

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Letters to the Editor

Sir:

We refer to the paper "Adsorption of β-Carotene: II. On Cation Exchanged Bleaching Clays," by K.Y. Liew, S.H. Tan, F. Morsingh and L.E. Khoo (*JAOCS* 59:480 [1982]), in which the authors make the statement:

> "In addition to small amounts of quartz and mica, Filtrol and Fulmont clays contain a substantial amount of kaolinite."

The authors base this statement on their interpretation of the X-ray powder diffractogram for Filtrol 105. Later in the article, they explain the apparent disappearance of kaolin from the X-ray powder diffractogram of ion-exchanged Filtrol 105 as follows:

> "After cation exchange and heating to 450 C, the 3.52Å d spacing in Filtrol and Fulmont disappeared, indicating that the kaolinite mineral in these clays had become amorphous."

We must respectfully disagree with the authors concerning their interpretations regarding Filtrol 105 bleaching clay. In fact, it contains no observable kaolinite – and certainly no "substantial amounts" of this material.

We believe the authors have misassigned their 3.52Å peak to kaolinite (3.58Å) when, in fact, it should have been assigned to the 3.50Å anhydrite ($CaSO_4$) peak. Anyhydrite, we know, is present as a minor constituent. As further evidence that this is the correct interpretation, we note: (a) that kaolinite should have an equally intense basal peak at 7.15Å which is, in fact, absent from their pattern and (b) that a second, low-intensity anhydrite peak at 2.86Å is present.

Our explanation for the disappearance of the 3.50Å peak is that it occurs by simple dissolution of the slightly soluble anhydrite phase during the washing steps performed by Liew et al., prior to ion exchange. In Figure 1, we show three X-ray powder diffraction patterns for Filtrol 105. Sample A has been dried to 100 C (but not washed). Sample B has been heated to 450 C for 4 hr (but not washed), and sample C has been washed, filtered, then dried at 100 C.

Clearly, the disappearance of the 3.50Å peak is not due to a thermally induced transition to an amorphous material as suggested by Liew et al. since sample B (calcined at 450 C for 4 hr) remains unchanged from sample A (dried at 100 C for 2 hr). Furthermore, sample C (washed and dried)

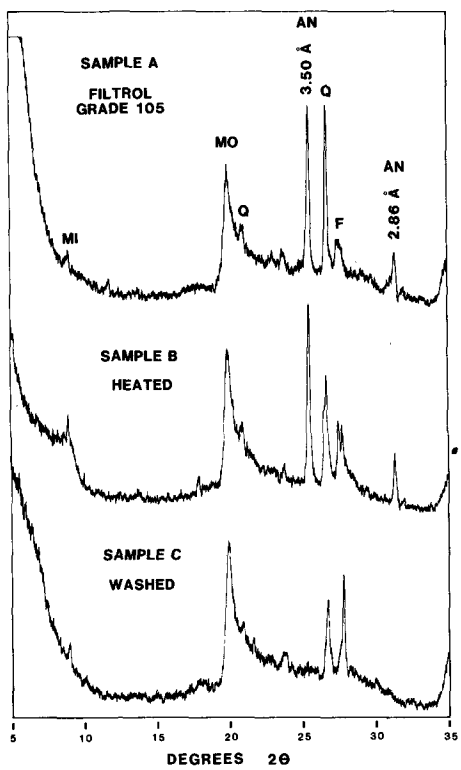

FIG. 1. X-ray powder diffractograms of Filtrol 105. Sample A dried at 100 C/2 hr; sample B heated (calcined) at 450 C/4 hr; sample C washed, filtered, dried at 100 C/2 hr (MI-mica, MO-montmorillonite, Q-quartz, AN-anhydrite, F-feldspar). Diffractometer conditions: Cu Kα radiation at 40 kV and 20mA, a range of 500 cps and a rate of 1C/min.

shows convincingly that simple washing and drying is quite sufficient to remove the anhydrite peaks (3.50Å, 2.86Å spacings) from the X-ray diffraction pattern.

Because anhydrite, a byproduct of the acid-activation process, is an intense reflector of X-rays at the 3.50Å spacing, relatively small amounts of this material give a quite noticeable peak when mixed with montmorillonite. We estimate its amount at 5% or less in a typical sample of Filtrol 105.