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Preliminary Studies on Processing of Sunflower Seed to Obtain Edible Protein Concentrates

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ABSTRACT

A process for obtaining edible-grade sunflower protein concentrate has been standardized. Dehulling the seed and removing polyphenols were accomplished using a centrifugal sheller and treating the kernels with acidified sodium chloride solution. The sunflower protein concentrate had a protein content of 60%, available lysine of 3.4 g/16 g N and was free from polyphenols. The protein efficiency ratio (PER) of the concentrate was 2.4 and in vitro digestibility was 90%.

INTRODUCTION

Sunflower cultivation was introduced on a national scale in 1971-72 as an oilseed crop in India. Since then considerable progress has been made by various states to promote this crop. The current estimate of production is nearly 300,000 MT on 720,000 ha (1). Sunflower meal is rich in protein and free from antinutritional factors (2). Chlorogenic acid and caffeic acids are the 2 polyphenolic constituents present in sunflower proteins that have undesirable effects, not only from the standpoint of nutrition, but also from that of technical application (3). Specifically, the polyphenolic oxidation products bring about an irreversible blocking of ϵ-amino and sulfhydryl groups, inactivating the gastrointestinal enzymes and reducing the biological value of proteins. In addition, during the production of protein concentrates and isolates from oilseeds, the properties that determine the utility value of the proteins are negatively influenced by the covalent bonding of oxidized phenolic acids to proteins and impart a dark green or yellow color to the product (4). Various methods have been reported in the literature for removing polyphenols from sunflower seed (5-14). However, most of these methods suffer from a combination of incomplete extraction, loss of proteins or the use of expensive reagents. In this paper, a method using acidic sodium chloride solution to extract polyphenols is described and the subsequent nutritional evaluation of the resultant processed meal is presented.

EXPERIMENTAL PROCEDURES

Precleaning

Black hybrid (EC-68414) sunflower seeds produced by Agro Seeds Corporation, Mysore, had 8-10% moisture content, and contained shriveled and unfilled seeds and other extraneous matter. The seeds were dried at 40 C in a through-flow dryer for 30 min to bring the moisture content to 4%. After cooling, they were passed through air-classifier equipment to remove unfilled, shriveled and lighter seeds.

Dehulling

After initial cleaning, the seeds were graded using BS-3 and BS-5 screens to separate small, medium and large seeds. These graded seeds were dehulled by a centrifugal sheller followed by air-classification. The sheller consisted of a circular metal housing lined with a hard rubber and PVC ring and had an inlet at the center for feeding in sunflower seed. The material fell on an impeller, which hurled the seed in a curved path against the rubber lining of the sheller by centrifugal force. As a result of the impact, the seeds were dehulled. The sheller housing had an 8-in. diameter and the impeller speed was 3,300 rpm. It had a capacity of dehulling 150 kg of seed per hr.

Partial Defatting

Kernels obtained after dehulling and air-classification were pressed in a laboratory Carver press at 5,000-6,000 psi for 2 hr and ca. 30% of the total oil was recovered. The resultant partially defatted kernels were brushed on a wire-mesh screen (30 mesh) and the translucent layer (testa) was removed by air-classification. The kernels free from the translucent layer were used for further processing.

Acidic Sodium Chloride Extraction

Three extractions of 100 g samples of partially defatted kernels were conducted at room temperature in plastic buckets with continuous slow stirring. The water-to-kernel ratio was 12:1 (V/W) with 1% NaCl for 2nd extraction and 2:1 (V/W) for 3rd extraction with water only. During these extractions, protein losses were minimized by adjusting the pH to 5.0 with 0.01 N HCl. Second and third washings were reused for fresh extraction of kernels. The extracts were separated from the kernel slurries by cloth filtration after each extraction. For comparison studies, water was used as an extractant for the partially defatted kernels. The wet, washed kernels were dried at 50 C for 40 min in a through-

flow dryer. The dried kernels were flaked to 0.01 in. thickness in a flaking roller and the flakes were solvent-extracted using food-grade hexane as solvent (4 extractions). The resultant defatted meal was thoroughly desolventized and ground to pass through 60-mesh screen.

Bench scale trials were also done using 25 kg/batch. Thirty-mesh screen with a shaker attachment was used for filtration.

Analytical Methods

The concentrations of chlorogenic acid (CGA) and caffeic acid (CA) in the defatted flour were analyzed according to the procedure of Pomenta et al. (15). The procedure of Srinivasan et al. (16) was used for quinic acid (QA) estimation. Moisture, protein, fat, crude fiber, ash and total solids were determined according to AOAC procedure (17). Amino-acid analysis was performed according to the microbiological assay of amino acids by Barton Wright (18). Available lysine was determined by Carpenter's procedure (19), in vitro digestibility studies performed by the procedure of Villegas et al. (20) and nutritional studies were done according to Campbell's procedure (21).

RESULTS AND DISCUSSION

Dehulling

Sunflower seeds procured and used in this study were found to be a mixture of small, medium and large seeds. Trials carried out using the centrifugal sheller indicated that the use of graded and optimally dried seeds having 4-5% moisture content were satisfactorily dehulled. Various dehullers, e.g., Diaf-grinder, underrunner sheller and the flour mill yielded 55-60% dehulled seed in the first pass. Recycling the uncut seeds was therefore necessary. By repeated passing, we found that breakage of the kernels as well as pulverization of both husk and kernel occurred, resulting in the formation of fines. However, in the centrifugal sheller, dehulling was accomplished in a single pass with 96% uniform dehulling and minimum breakage of kernels. The hull content was 25%.

The chemical composition of defatted meals (not dehulled and dehulled) prepared in the laborary by direct solvent extraction, is shown in Table I. As expected, the dehulled sunflower meal had a high protein content (53.3%) and low crude-fiber content (8.3%) compared with the not dehulled sunflower meal, which had a lower protein content (30%) and higher fiber (21.4%).

Extraction Efficiency

The pressed kernels were leached with salt solution. The partial defatting with the Carver press, which was mentioned previously, was necessary for quick penetration of the NaCl solution into the aleurone layer during acidic NaCl treatment. A gradual decrease of the NaCl concentration and further water washing helped, by osmosis, in the complete removal of CGA. Sosulski et al. (22) have shown that the separation of the translucent layer or testa from the kernels was essential for the rapid diffusion of CGA from sunflower. Although the translucent layer constituted only 3% of the kernel weight, its removal reduced crude-fiber content of the meal drastically (Table I). Aliquots of washed kernels were taken during each extraction, dried, solvent extracted and analyzed for protein and polyphenol contents. Experiments with batch extraction, using various solvent-to-meal ratios, demonstrated that a minimum of 2 extractions were necessary to remove 95% or more of the polyphenols in sunflower kernels. Complete removal of CGA was not achieved in a single extraction and repeated extractions were necessary to achieve even a partial removal. The hydrogen bond between the hydroxyl groups of phenolic compounds and peptide bonds in the protein is known to be unusually strong and equilibrium in aqueous solutions strongly favors the formation of complexes between phenols and proteins (23).

TABLE I

Proximate Composition and Polyphenol Content of Defatted Sunflower Flour

Constituents	Not dehulled	Dehulled		
		Control	Water-treated	NaCl treated
Protein (N X 6.25)	30.1	53.3	57.3	59.7
Fat (%)	1.0	1.2	1.0	1.0
Crude fiber (%)	21.4	8.3	2.3	2.1
Ash (%)	6.1	5.8	4.5	3.4
Polyphenols (%)				
Chlorogenic acid	0.93	2.0	0.74	ND[a]
Caffeic acid	0.58	0.82	0.20	ND[a]
Quinic acid	0.36	0.30	0.05	ND[a]

[a]Not detectable.

In the 1st extraction with 2% NaCl solution, 79% of the total polyphenols were removed, including 74% of the CGA (Table II). In comparison, the kernels extracted with water showed only a 46% extraction of the total polyphenols, including 40% of the CGA. The 2nd extraction, with 1% NaCl solution, almost completely removed the residual polyphenols present in kernels and yielded polyphenol-free kernels. The 3rd extraction was only to wash any traces of salt, fiber and the like from the kernels. Three extractions of the kernels with water removed 63% of the total polyphenols (1.93 g/3.06 g) and produced a low CGA-containing product (Table II). The combined NaCl extracts (1,900 mL) contained 28 g soluble solids (including 3 g of polyphenols), compared with 14.5 g of soluble solids (including 1.93 g polyphenols) in the case of the water extracts. Protein loss was only 7% in NaCl-extracted kernels compared with 2.5% in water-extracted kernels. Taha and El-Nockrashy (13) have reported 25% protein loss and 95% removal of coloring matter in a water wash at pH 4.0 with solvent; the meal ratio was 50:1. Most of the soluble sugars were removed during extraction.

As shown in Tables I and II, the quantities of CGA and other polyphenols solubilized in each successive extraction of the kernels were substantially decreased. Reusing the solvents in a systematic manner and improving the extraction efficiency of successive kernel extraction while reducing solvent quantity appears logical. The total time taken for complete extraction using NaCl was only 90 min. The sunflower flour did not give any color under alkaline conditions and the protein isolate obtained by using acidic NaCl-extracted flour (defatted) was white in color.

The chemical composition of sunflower protein concentrate also is shown in Table I. Acidic NaCl treatment increased the protein content from 53–60%. Loss of protein during extraction was ca. 7% and removal of soluble sugars and other constituents enhanced the protein content. Fat loss during leaching in both water and acidic NaCl extractions was only 2-3%. Removal of testa from the kernel helped reduce crude fiber content to 2.1%. Removal of soluble solids, interfering substances and traces of NaCl helped in reducing the ash content to ca. 3%. The resultant flour was completely free of CGA, CA and QA.

The available lysine content of 3.85 g/16 g N (Table III) shows that no binding occurs of ϵ-amino groups to phenolic groups of polyphenols during processing.

TABLE II

Comparative Efficiency of Extracting Sunflower Kernels with Water and Sodium Chloride Solutions

Extraction	Solids removed	Protein loss	Polyphenol extracted[a]	Polyphenol still present	Solids removed	Protein loss (%)	Polyphenol extracted[a]	Polyphenol still present
	Water (g/100 g)				NaCl with 0.05 N HCl (g/100 g)			
I	11.8	1.7	CGA 0.80	1.18	15.0	3.3	CGA 1.46	0.52
			CA 0.56	0.26			CA 0.72	0.10
			QA 0.06	0.20			QA 0.23	0.03
			1.42	1.64			2.41	0.65
II	1.7	0.6	CGA 0.20	0.98	10.0	0.7	CGA ACR[b]	ND[c]
			CA 0.10	0.16			CA ACR[b]	ND[c]
			QA 0.10	0.10			QA ACR[b]	ND[c]
			0.40	1.24				
III	1.0	0.1	CGA 0.11	1.13	3.1	0.2		
			CA 0.11	1.13	3.1	0.2	...	ND[c]
			QA 0.11	1.13	3.1	0.2		
Total (%)	14.5	2.4	1.93	1.13	28.1	7.0	...	...

[a]CGA—chlorogenic acid; CA—caffeic acid; QA—quinic acid.
[b]ACR—almost completely removed.
[c]ND—not detectable.

TABLE III

Essential Amino Acid Composition of Sunflower Seed Protein

Amino acid	Literature values (g/16 g N)	Defatted flour	NaCl-extracted flour
Lysine	3.4–4.2	3.83	3.80
Methionine	1.7–2.1	1.85	1.78
Cystine	1.6–2.2	1.60	1.64
Tryptophane	1.0–1.2	1.10	1.22
Threonine	3.0–3.4	3.60	–
Leucine	6.0–6.2	6.8	6.40
Isoleucine	3.90–4.1	6.10	6.00
Valine	4.30–5.1	5.10	4.85
Phenylalanine	4.6–4.8	5.20	4.80
Tyrosine	2.6–2.8	–	–
Available lysine		3.38	3.40

Nutritive Value

Sunflower proteins have a much lower lysine content than legumes and animal proteins (20). However, sunflower proteins are rich in other essential amino acids, especially the sulfur-containing amino acids (Table III). The protein efficiency ratio (PER) of sunflower flour (both not dehulled and dehulled and defatted) and concentrate (polyphenol free) were evaluated with casein as the control in rat-feeding trials (Table IV). Average meal consumption and weight gain of sunflower flour (dehulled) exceeded those obtained with sunflower flour (not dehulled). PER was significantly higher in dehulled flour than in not dehulled flour. Lysine supplementation (0.2%) to sunflower flour (dehulled) improved the feed consumption, weight gain and PER of the sunflower flour significantly more than the unsupplemented flour and undehulled flour. Polyphenol-free concentrates gave a value of 2.39, the same as that of lysine-supplemented flour (dehulled). When the concentrate was supplemented with lysine (0.2%), the weight gain, feed consumption and PER were significantly higher than the unsupplemented sunflower-protein concentrate and casein diets.

TABLE IV

Nutritional Studies on Sunflower Protein

Group[a]	Protein source and level of protein	Average initial weight (g)	Average protein intake (g)	Average gain in weight (g)	PER[b]
A	Not dehulled, defatted flour (10.09% protein)	33.4	20.6	35.6	1.72
B	Dehulled, defatted flour (9.6% protein)	33.4	23.2	47.0	2.02
C	B + 0.2% l-lysine HCl (10.09% protein)	33.5	21.6	52.0	2.40
D	Dehulled, NaCl-extracted, defatted flour (9.6% protein)	33.9	22.0	52.6	2.39
E	D + 0.2% l-lysine HCl (10.0% protein)	35.9	22.0	56.8	2.57
F	Casein (10.4% protein)	33.5	21.9	53.8	2.45

[a]Ten male rats in each group, randomized block design, 4 weeks duration, 10% protein in diet.
[b]PER, standard error of mean ±0.048.

In vitro Digestibility

Literature studies have shown that sunflower protein is highly digestible and possesses a high biological value (2) and conforms to the value obtained for sunflower flour. Polyphenol-free concentrate showed much high value (90%) than that of the sunflower flour (84.7%).

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Phosphatidyl Ethanolamine as a Synergist for Primary Antioxidants in Edible Oils

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ABSTRACT

Dipalmitoyl phosphatidyl ethanolamine (DPE) is a potent synergist for a wide range of primary antioxidants in edible oils at elevated temperature, i.e., above 80 C. At lower temperatures it has very little synergistic action. At 120 C the synergistic effect increases progressively as the concentration of synergist increases from 0.025% to 0.25%. At a given level of synergist, its effect is proportionately greater at low rather than high levels of primary antioxidant.

INTRODUCTION

Though without significant antioxidant properties themselves, at elevated temperatures some of the naturally occurring classes of phospholipids (PL), in particular phosphatidyl ethanolamine (PE), greatly enhance the activity of primary antioxidants in edible oils with limited stability. Phosphatidyl choline and phosphatidyl serine are also effective, but markedly less so, and phosphatidyl inositol is without synergistic activity (1-3).

PE has been clearly shown to act synergistically in cooperation with various polyhydroxy flavones and flavanones (1), with polyhydroxy isoflavones (2) and with tocopherols and some of their derivatives (3). In these studies both lard, which contains no natural primary antioxidants, and soybean oil, which contains several tocopherols, have been studied. Assessment of stabilizing effects has been, as is customary, by measurement of induction periods under accelerated storage conditions, at 100-140 C.

From these earlier studies, the expectation that PE would be capable of providing synergistic effects with all primary antioxidants and polyunsaturated substrates seemed reasonable, but this needed to be demonstrated with a wider range of primary antioxidants. Further, some quantitative aspects, e.g., the relationships between concentration of synergist and the magnitude of the antioxidant effect, needed to be established. Finally, the influence of temperature on the antioxidant effect was obscure. The present communication reports the results of our studies on these themes.

EXPERIMENTAL PROCEDURES

Materials

Lard was donated by Messrs. Scot Bowyers Ltd., Trowbridge, England. It was unrefined, had not been chemically processed and was free from added antioxidants. Refined, low-erucic rapeseed oil was donated by Croda Edible Oils Ltd., Hull, England.

sn-Dipalmitoyl phosphatidyl ethanolamine (DPE) of 98% purity was purchased from Sigma (London) Ltd., England, and dl-α-tocopherol was kindly donated by Roche Products, Dunstable, England.

The other primary antioxidants were of best commercial quality when available. Propyl caffeate was prepared by direct esterification of caffeic acid with n-propanol in the presence of a trace of H_2SO_4. The product was recrystallized from aqueous ethanol. The preparation of 3,4-dihydroxy chalcone was as described in our earlier communication (4).

Methods

Induction periods were determined in the automated Rancimat (Metrohm AG, CH-9100 Herisau, Switzerland) at defined temperatures in the range 60-140 C. A continuous airstream is passed through the heated sample and the volatiles passed into a conductivity cell. Conductivities are