♣Effect of Heat Processing on Phenolic Constituents and Nutritional Quality of Sunflower Flours

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ABSTRACT

Heat processing of dehulled, defatted sunflower flour resulted in a reduction in phenolic constituents, the destruction of chlorogenic acid (CGA) being more than that of caffeic acid (CA) and quinic acid (QA). Considerable losses in the available lysine rather than total lysine content were observed when sunflower flour was autoclaved at 1 kg/cm^2 (120 C) for periods varying from 5 to 60 min. The N solubility of sunflower protein in 0.02N NaOH showed a progressive decrease as the duration of heat treatment increased. Moderate heat treatment (15 min at 120 C) of the flour had no significant beneficial effect on in vitro digestibility. Any advantage in decreasing the levels of CGA, CA and QA contents by moderate heat treatment apparently was lost by the decrease in available lysine content, thus lowering the protein efficiency ratio (PER) from 1.97 to 1.71 and Net Protein Utilization (NPU) from 62.7 to 54.8, respectively.

INTRODUCTION

Sunflower *(Helianthus annuus)* is a newly introduced oilseed crop in India, and commercial processing of the seed for oil has been in progress for 10 yr. Sunflower proteins are reported to have good nutritional value and digestibility. They are, however, deficient in lysine (1). Even though sunflower has no major antinutritional toxic factors, it contains certain phenolic compounds which cause discoloration of the flour and the protein products (2). Chlorogenic acid (CGA), which constitutes about 70% of total polyphenols, is known to affect digestibility and the availability of lysine and methionine in sunflower flour (3,4). Various methods have been described for removal of polyphenols, involving the use of aqueous, organic solvents and high temperatures (4-8). Heat processing is one of the methods for complete removal or inactivation of toxic principles such as trypsin inhibitors and hemagglutinins which influence the nutritional value of food proteins (2). The deleterious effects of high processing temperatures on the nutritive value of sunflower protein with a special reference to the destruction of total lysine has been studied by many workers (9-12). Since lysine is the limiting amino acid in sunflower protein, further lysine loss during processing will decrease the nutritive value. The objective of the following studies was to determine the effect of heat processing of dehulled and defatted (by direct solvent extraction using hexane) sunflower flour upon (i) CGA and its hydrolyzed products, namely caffeic acid (CA) and quinic acid (QA), and (ii) nutritional quality of unheated and heated sunflower flours.

MATERIALS AND METHODS

Preparation of Sunflower Flour

A Russian variety of sunflowerseed (EC 68415) was procured from Karnataka Agro Seeds Corp., Mysore. It was cleaned, graded and dehulled by centrifugal sheller according to the method of Shamanthaka Sastry (8). Dehulled seeds were flaked to 0.25 mm thickness using twin flaking rolls and solvent extracted using food grade hexane. The extracted flour was dried at room temperature for 24 hr and finally dried in a vacuum shelf drier for 4 hr to remove traces of solvent. This flour was used in all the following experiments.

Heat Processing

Sunflower flour was subjected to heat treatment as follows: It was spread in stainless steel trays to a thickness of 0.5 cm, covered to minimize moistening with condensed steam and autoclaved at 1 kg/cm^2 (120 C) for 5, 15, 30, 45 and 60 min, respectively, then air dried to uniform moisture levels. The samples were ground to pass through 60-mesh screen and taken for analysis.

All the samples were analyzed for their chemical constituents by standard procedures of the AOCS (14). Total lysine was estimated in these samples by microbiological assay procedure (15) and available lysine by Carpenter's procedure (16). Chlorogenic acid and caffeic acid were determined according to Pomenta and Burns (17) and quinic acid by the method of Srinivasan and Sprinson (18). Nitrogen solubility was studied by the procedure of Lyman et al. (19).

In vitro digestibility was carried out according to the method of Villegas et al. (20). Controls without enzyme were run concurrently in each case. In addition, digests were examined for the presence of any protein intermediary products stable to enzyme attack by the trichloroacetic acid (TCA) precipitation method of Birk and Bondi (21). For this, the enzymatic digests were treated with an equal volume of 10% TCA to precipitate the undigested protein, centrifuged, and the nitrogen determined in the supernatant.

Nutritional studies were done by the procedures of Campbell (22). Data were analyzed by Duncan's new multiple range test (23) to locate significant differences.

RESULTS AND DISCUSSION

Data on the chemical composition and phenolic constituents present in sunflower flour is given in Table I. Percentage destruction of phenolic constituents by autoclaving the flour for various periods are given in Table II. The protein and CGA contents of 58.8% and 1.41%, respectively, were high. Autoclaving for a period of 5 and 15 min at 120 C resulted in a loss of 47% CGA, while at the end of 60 min the loss was 85.8%. The losses in the case of CA ranged from 7% to 44% and in QA from 3.7% to 33.8% during five 60-min heat treatments. These studies have indicated that

TABLE I

Approximate Composition of Sunflower Flour

	%
Protein (N X 6.25)	58.8
Fat	0.9
Crude fiber	9.6
Ash	7.6
Chlorogenic acid	1.41
Caffeic acid	0.72
Quinic acid	0.33
Total phenolic constituents	2.45

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TABLE II

Effect of Autoclaving at 1 kg/cm² (120 C) on Phenolic Constituents in Sunflower Flours

Duration of autoclaving	CGA	% loss	CA	% loss	QA	% loss	Total phenolic constituents	% loss
–	1.41	–	0.72	–	0.33	–	2.45	–
5 min	0.75	47.0	0.88	–	0.30	–	1.93	21.6
15 min	0.75	47.0	0.67	7.0	0.31	3.7	1.72	29.8
30 min	0.50	64.5	0.53	27.0	0.31	4.6	1.34	45.6
45 min	0.25	82.0	0.48	34.0	0.27	15.0	1.00	59.2
60 min	0.20	85.8	0.40	44.4	0.21	33.8	0.82	66.8

TABLE III

Effect of Autoclaving at 1 kg/cm² (120 C) Upon the Total and Available Lysine Contents in Sunflower Protein

Duration of autoclaving	Total lysine g/16 g N	% loss	Available lysine g/16 g N	% loss
–	3.83	–	3.12	–
5 min	3.77	1.6	2.91	6.7
15 min	3.76	1.8	2.83	9.3
30 min	3.60	6.0	2.52	19.2
45 min	3.50	8.6	2.33	25.3
60 min	3.30	13.8	2.08	33.3

CGA was more susceptible to heat treatments. Milic et al. (9) found that heating sunflower kernels to 100 C for 5 hr decreased CGA by 43% and increased QA by 44%, a value similar to the decrease of CGA in their study. However, the present results show only a progressive decrease in QA content as a result of autoclaving. CA was not produced by hydrolysis of CGA. The results in the present studies, however, showed that there was no increase in QA content as a result of heat treatment.

Data presented in Table III show that the total lysine content of sunflower flour was 3.83 g/16N, and autoclaving for up to 15 min did not reduce the total lysine content appreciably. Increasing the time of autoclaving from 45 to 60 min resulted in losses of 8.6% and 13.8%, respectively. The extent of loss of total lysine in the present study is, however, much lower than the value reported by Alexander and Hill (10). They reported that solvent extracted meals after being heated in an autoclave at 118 C/1 hr and 2 hr contained 21% and 57% less lysine, respectively, than the original flour. Bendemer and Evans (13) also have observed that when sunflowerseeds were heated in an oven at 250 C for 45 min, the total lysine content decreased from 3.6 g to 2.1 g (58% loss).

Comparative data on the effect of heat processing of sunflower flour on the available lysine content are meager (3,4). Data presented in Table III show that, in contrast to the relatively low percentage loss of total lysine, loss of available lysine was much higher during heat treatment. At the end of 30 min and 60 min, the percentage losses in available lysine contents were 19.2 and 33.3%, respectively. Any possible advantage in reducing the levels of CGA, CA and QA content by heating were negated due to the loss of this essential amino acid.

Data given in Table IV show that the dehulled, defatted sunflower flour gave a maximum protein solubility of 96.9% at a flour: solvent ratio of 1:50. When the flour was autoclaved at 120 C for different periods of time, there was a progressive decrease in nitrogen solubility indicating that the proteins were greatly denatured.

The in vitro digestibility value of sunflower flour (Table IV) was 82%, comparable to the value reported by others (2,3,6). The flour autoclaved at 120 C for 5 and 15 min gave digestibility values of 86.1% and 88.8%, respectively. There was no significant improvement in digestibility following such moderate heat treatment. However, autoclaving at 120 C for 60 min decreased the digestibility value to 82.0%. Perhaps due to the heat treatment, the action of polyphenols and quinones on the ε amino groups of lysine

TABLE IV

Enzymatic Digestibility and Nitrogen Solubility of Proteins in Sunflower Flours

Duration of autoclaving	Digestibility (Sol N/Total N X 100)		TCA extract % N		N Solubility %
	Without enzyme	With enzyme	Without enzyme	With enzyme	
–	33.7	81.9	29.3	81.2	96.9
5 min	13.8	86.1	11.7	82.6	67.9
15 min	112.7	88.8	11.7	82.6	44.7
30 min	13.1	85.9	11.7	83.3	32.6
45 min	15.8	84.7	12.4	82.6	31.6
60 min	13.4	82.0	11.7	82.4	5.7

TABLE V

Effect of Heat Processing on the Nutritive Value of Sunflower Flour

Diet	Gain in wt g	Diet intake g	Protein intake g	Protein efficiency ratio	Feed:Gain ratio	Gain in wt g	Net protein utilization
Sunflower flour 10.0% protein	47.05	241.80	24.18	1.93 x	5.14 x	21.81	62.70 x
Sunflower flour autoclaved at 120 C (15 min) 10.3% protein	49.05	282.20	29.07	1.71 y	5.72 y	24.62	54.77 y
Casein 10.3% protein	53.75	212.50	21.90	2.46 z	3.96 z	16.88	68.61 z
SE_m (1 8df)	–	–	–	±0.05	±0.13	–	–
SE_m (14 df)	–	–		–	–	–	±1.47

Means of the same column followed by different letters differ significantly according to Duncans' New Multiple Range Test ($P < 0.05$).

and subsequent polymerization of the quinones into polyphenol protein complexes could render some of the essential amino acids inaccessible to the digestive process of monogastric animals, causing a decrease in digestibility and nutritive value. Horigome and Kendatsu (3) observed that when casein interacted with CGA or CA under oxidation conditions, its biological value was decreased.

The TCA soluble fraction of the enzymatic digests contained practically all the nitrogen present in the enzymatic digest (Table IV). This would indicate that no enzymatically resistant protein fractions were present in sunflower flour and other autoclaved flours.

Data on the PER values of the proteins of sunflower are given in Table V. Sunflower flour gave a PER value of 1.96. When the flour was autoclaved at 120 C for 15 min there was a reduction in the PER value from 1.96 to 1.71, though the gain in weight of rats fed on the heat-processed flour diet was nearly the same as that of the control rats. However, the protein intake of rats fed on the heated sunflower flour diet was significantly higher (29.1 g) than casein (21.9 g).

The data on the feed efficiency ratio of rats also suggest the presence of some of the heat sensitive material in sunflowerseed which was destroyed or inactivated by moderate heating. Destruction or absence of this component then permitted improved animal performance. Some of this improved performance might be attributed to changes in the levels of CGA, CA and QA. Milic et al. (9) also have indicated that CGA was an effective trypsin inhibitor, but QA had no effect on digestion by trypsin in in vitro enzymatic studies. Destruction of CGA by autoclaving sunflower flour may have permitted greater utilization of the diet containing sunflower flour. The work of Amos et al. (11) also indicated that moderate heat treatment results in better growth rates and improved feed efficiency ratios than was obtained with unheated flour.

Generally, phenolic compounds can combine with proteins reversibly by hydrogen bonding and irreversibly by oxidation to quinone followed by covalent condensation (4). Shamanthaka Sastry (13) has observed that the 11S protein (major protein fraction [70%] in sunflowerseed) binds CGA/CA/QA essentially through hydrogen bonding. With an increase in temperature, binding of CGA/CA by the 11S protein decreased. In fact, binding was totally absent at 55 C. Temperature had an adverse effect on formation of hydrogen bonds. Eklund (25) has demonstrated that the PER, biological value and digestibility of the casein diet containing 1% (w/w) of CGA did not differ from values for the corresponding casein diet free from CGA. No change was observed in the nitrogen balance of the experimental rats. Perhaps the decrease in the PER of autoclaved sunflower flour is due to the decrease in the available lysine content, which was due, in turn, to the oxidation of CGA to quinone followed by covalent condensation with ϵ amino group of lysine during autoclaving.

Using samples of unheated and heated sunflower and a casein control, it was possible to demonstrate the adverse effects of heat treatment on protein nutritive value. The NPU value of 54.7 for heated sunflower was significantly lower than that of the sunflower and casein controls.

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