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EFFECT OF HEAT PROCESSING ON NITROGEN SOLUBILITY AND DIGESTIBILITY OF PROTEIN IN SUNFLOWER MEAL

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Proximate composition of seven varieties of sunflower seed and meal and their nitrogen solubility profile in water (20.5-22.5%), 5% NaCl (49.6-52.8%), 70% C_2H_5OH (3.0-4.0%) and 0.2% NaOH (9.8-11.0%) was determined. *In vitro* digestibility of untreated and enzymic treated sunflower meal was found to be 34.5% and 83.5% respectively. The autoclaving of untreated meal at 1kg/cm^2 for 5-60 mins., showed a gradual decrease *in vitro* protein digestibility from 34.5 to 12.0%. *In vitro* protein digestibility of enzymic treated meal increased from 83.5 to 87.2% after 15 mins. autoclaving. Further increase in autoclaving time to 60 mins showed a gradual decrease in the *In vitro* digestibility. Hence processing of sunflower meal at 1 kg/cm^2 for 15 mins. was found most suitable.

Key words: Autoclaving, Nitrogen solubility, Digestibility.

Introduction

The high cost and limited availability of animal proteins in deficient areas and the increasing realization that oilseeds hold potential to bridge the protein gap in many countries of the world, have stimulated a great deal of interest among food scientists. In recent years, sunflower seed has become an important oilseed crop because it is well adopted to the climatic conditions of Pakistan. The sunflower seeds contain 40% high quality oil which is a rich source of essential fatty acids [1]. The seed cake left after oil extraction contains 35-40% protein with a well balanced amino acid profile [2]. Sunflower seed meal i.e. defatted cake, has a higher content of good quality protein than cereals which are still the main source of protein in many countries. Sunflower seed meal has limited use in animal and poultry rations due to the presence of antinutritional factors i.e. crude fibre, phytic acid and polyphenols [3-5]. Many researchers have reported different techniques to improve the nutritional value of the meal [6-9].

The present study was carried out to investigate the effect of heat processing on nitrogen solubility and digestibility of protein in sunflower meal.

Experimental

Seeds of three varieties of sunflower (*Helianthus annus* L.) were procured through the courtesy of Punjab Seed Corporation Limited, whereas other four varieties were collected from different places of the local market. The clean and dirt free seeds were dehulled using a locally made dehuller and separator.

Processing. PCSIR-IDRC Model Oil Expeller was employed for extraction of oil. 20 kg. sunflower seed kernels obtained after dehulling and hull separation were pre-pressed twice by keeping a distance of 12 mm between screw and cone followed by defatting of cake with *n*-hexane in Soxhlet extractor to reduce the oil content to minimum of 2 ± 0.5%. The sunflower meal i.e. defatted cake was ground to 80 mesh size.

Heat treatment. Sunflower meal was subjected to heat treatment as follows:

The triplicate samples of meal (80 mesh) were spread in stainless steel trays (30 x 45 cm) to thickness of 0.5 cm, covered with polythene sheet to minimize moistening with condensed steam and autoclaved 1 kg/cm^2 (120°) for 5, 15, 30, 45 and 60 mins. respectively, then air dried to uniform moistures levels.

Nitrogen solubility profile of the sunflower meal after processing was determined to see the change in the extractibility of different proteins soluble in water (albumin) 5% NaCl (globulin) 70% ethyl alcohol (prolamin) and 0.2% NaOH (glutelin). The protein digestibility (with and without enzyme) was also determined to further confirm the change in quality of protein.

Nitrogen solubility profile. In order to determine the nitrogen solubility profile of sunflower meal protein, the successive extraction media were distilled water, 5% sodium chloride, 70% alcohol and 0.2% sodium hydroxide [6]. All procedures were conducted at room temperature except ethanol extraction which was carried out at 65° in a water bath. The initial size of sample was 2 g of meal and at each stage the residue from the previous extraction was shaken with 25 ml of solvent for 15 mins, centrifuged at 3,000 r.p.m. for 15 mins., and the supernatant decanted. Two successive extractions were made with each solvent and the peptizate combined for the determination of soluble nitrogen. The nitrogen content of

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the extracted and final residue were determined by micro-Kjeldhal procedure [7]. The extracted nitrogen was expressed as the percentage of total meal nitrogen.

The sunflower meal (2 g) was also extracted with 0.25% aqueous sodium sulphite at pH 10.5 [8]. The extraction was carried at room temperature for 1 hr. with constant stirring using a meal water ratio 1:50; (w/v).

In vitro protein digestibility: In vitro protein digestibility of sunflower meal containing 52.5% protein was determined (with and without enzyme) according to the method of Mandal, *et al.*, [9] as outlined below:

Sunflower meal (100 mg) was mixed with 2 mg suspension of pepsin (Sigma Chemical Co.) in 5 ml dil HCl (pH 2.0) and incubated at 37° for 16 hrs. 2 ml of 0.5% pancreatin (Sigma Chemical Co.) solution in 0.1 M borate buffer (pH 6.8) was added to the reaction mixture and again incubated at 37° for 24 hrs. followed by addition of 7 ml of 10% (w/v) trichloroacetic acid (TCA). The suspension was centrifuged at 5,000 r.p.m. for 15 mins. The residue was washed twice with 5% TCA solution. The supernatant were pooled and evaporated to dryness. Nitrogen content was determined by micro-Kjeldhal procedure and factor 6.25 was used to convert to crude protein [7]. The digestibility of each sample was calculated as the nitrogen in the sample supernatant minus nitrogen in the enzyme blank supernatant, expressed as percentage of nitrogen in starting mateial.

Analytical. The average chemical composition i.e. moisture, ash fat, crude protein and crude fibre contents of sunflower meal (three replicates) were determined according to AOAC methods [7], phytic acid in meal was estimated by the method of Wheeler and Ferrel [10]. The nitrogen free extract (NFE) was calculated as follows:

NFE (%) = 100 - (Crude protein + Fat + Crude fibre + Ash)

The data collected was statistically evaluated. The difference in mean values were tested by Duncan's Multiple Range Test [11].

Results and Discussion

The sunflower seeds collected from Punjab Seed Corporation and local market in 1991 contained 6.4-8.6% moisture, 21.4-25.8% crude protein, 29.8-34.6% fat, 10.6-12.6% crude fibre 4.0-5.0 ash, 2.5-3.4% phytic acid and 25.1-32.0% NFE (Table 1). The results showed that the seed varieties collected from Punjab Seed Corporation contained higher protein, and fat and lower crude fibre, phytic acid and nitrogen free extract (NFE) contents. The variation appeared to be due to the different seed varieties grown in different environmental conditions. The results are comparable with the findings of Klynchkin, *et al.*, [12] and Niazi, *et al.*, [3].

The proximate composition of sunflower meals obtained after pre-pressing of kernels followed by solvent extraction is shown in Table 2. The meals contained 4.5-6.2% moisture, 45.0-52.5% crude protein, 1.8-2.5% fat, 6.5-7.8% crude fibre, 6.2-6.8% ash, 4.0-4.5% phytic acid and 32.8-38.6% NFE. The significant increase ($P<0.01$) in the percentage of all the ingredients of the meals with respect to sunflower seeds (Table 1) was due to extraction of oil from the kernel fractions. The results are in agreement with the findings of other researchers [3, 4, 13] who reported that crude protein fat and phytic acid were centred in the kernel fraction whereas antinutritive polyphenols were found in hull faction of sunflower seeds. Thus removal of hull fraction was of prime importance.

The amount of nitrogen extracted from seven varieties of sunflower meals varied from 20.5-22.5% in water, 49.6-52.8% in 5% NaCl, 3.0-4.0% in 70% alcohol and 9.8-11.0% in 0.2% NaOH. The nitrogen left in the residues ranged in between 12.3-15.0% (Table-3). Almost half of nitrogen of meals (49.6-52.8%) was soluble in 5% NaCl and more than 40% of the remaining half was extracted by water (20.5-22.5%). The results are comparable with the findings of Sosulski and Bakal [6] who observed that sunflower meal proteins were primarily salt soluble but significant protein was extracted by initial water and final alkali treatment. The present results indicated that in contrast to earlier reports, summa-

TABLE 1. PROXIMATE COMPOSITION OF SUNFLOWER SEED.*

Source	Moisture (%)	Crude protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Phytic acid (%)	NFE** (%)
P.S.C.+	6.5	25.8	34.6	10.6	4.0	2.5	25.1
" "	6.8	24.2	33.8	11.2	4.4	2.5	26.4
" "	6.4	24.5	32.5	10.8	4.3	2.6	26.1
Local market	7.5	22.8	30.6	12.2	4.8	2.8	29.6
"	8.6	23.6	32.2	11.8	4.6	2.7	27.8
"	8.2	24.0	32.1	12.6	5.0	3.2	25.3
"	8.0	21.4	29.8	12.0	4.8	3.4	32.0

* Dry matter basis,** Nitrogen free extract, + Punjab Seed Corporation. – All values in the table represent average of three replicates.

rized by Smith [14], sunflower proteins are readily extracted from the defatted meals.

The effect of heat processing i.e. autoclaving at 1 kg/cm^2, on sunflower meal for 5, 15, 30, 45 and 60 mins. on its nitrogen solubility in Na_2SO_3 and *in vitro* digestibility is given in Table 4. The moisture content of the meal increased by 3.5-5.4% over the control after different autoclaving time. The increase in moisture content were due to condensation of steam. Untreated sunflower meal showed maximum nitrogen solubility of 87.7%. The autoclaving of the meal at 120° for different periods of time, showed a gradual decrease in nitrogen solubility to 60.5% and 22.8% after 5 and 60 mins., respectively. It clearly indicated that the proteins were greatly denatured when processed at 120° for longer time. The results are comparable to the values reported by other researchers [8-15], but 9.2% less than those reported by Shastry and Subramanian [5], perhaps it was due to the difference in seed variety and processing conditions.

The *in vitro* digestibility value of untreated sunflower meal (34.5%) and enzyme treated sunflower meal (83.5%) were comparable to the values reported by others [5, 15]. The autoclaving of untreated sunflower meal for 5 mins. decreased the digestibility from 34.5% to 15.9% (Table 4). Further increase in the autoclaving time (15-60 mins.) showed a gradual but non-significant decrease in digestibility (13.7-12.0%). Enzymic treated sunflower meal autoclaved for 5 and 15 mins. showed 85.5% and 87.2% digestibility, respectively. There was no significant improvement in digestibility of meal for allowing such moderate heat treatment. However, autoclaving at 120° for 60° mins. decreased the digestibility value to 80.0% perhaps the denaturation of protein and formation of polyphenol protein complexes rendered the heat labile and some of the essential amino acids inaccessible to the action of enzyme thus causing a decrease in digestibility [5, 15, 16].

The present investigation showed that a major portion of total extracted protein were soluble in water (20.5-22.5%) and in 5% sodium chloride (49.6-52.8%). The protein fraction soluble in water (albumin) and in sodium chloride (globulin) are most easily assimilated by non-ruminants e.g. broilers and layers [17]. Thus, pressence of large portion of albumin and globulin in sunflower meal would make it most suitable for incorporation in poultry rations. It is, thus, concluded that sunflower meal obtained after proper processing i.e. autoclaving the meal at 120° for 15 mins., has a great potential for its utilization in poultry rations as a substitute of costly vegetable and animal proteins.

TABLE 2. PROXIMATE COMPOSITION OF SUNFLOWER MEAL.*

Source	Moisture (%)	Crude protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Phytic acid (%)	NFE** (%)
P.S.C.+	5.8	52.5	2.0	6.5	6.2	4.0	32.8
"	6.2	48.2	2.5	7.6	6.5	4.5	35.2
"	5.5	51.2	1.8	7.2	6.4	4.2	33.4
Local market	5.0	47.5	2.2	6.9	6.2	4.0	37.2
"	4.5	45.0	1.8	7.8	6.8	4.0	38.6
"	5.2	46.8	2.1	7.5	6.8	4.2	36.8
"	6.0	49.4	2.4	7.0	6.4	4.4	34.8

*Dry matter basis, ** Nitrogen free extract, + Punjab Seed Corporation. – All values in the table represent average of three replicates.

TABLE 3. NITROGEN SOLUBILITY PROFILE OF SUNFLOWER MEAL.*

	Percent of total meal nitrogen soluble in				
Source	H_2O	5% NaCl	70% Ethyl alcohol	0.2% NaOH	% N in residue
P.S.C.+	22.5aa	50.4a	4.0a	10.8a	12.3a
"	21.0b	52.8b	3.2b	10.4b	12.6a
"	20.5b	50.5a	3.5c	10.5b	15.0b
Local market	21.9a	50.2a	4.0a	10.4b	13.5b
"	21.1b	51.2b	3.0b	10.1b	14.6b
"	22.0a	50.0a	3.8c	9.8b	14.4b
"	21.3b	49.6a	3.7c	11.0a	15.0b

*Dry matter basis, + Punjab Seed Corporation. – Means of the same column followed by different letters differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test. – All values of the table represent average of three replicates.

TABLE 4. EFFECT OF AUTOCLAVING ON DIGESTIBILITY AND NITROGEN SOLUBILITY OF PROTEIN IN SUNFLOWER MEAL.*

Duration of autoclaving (mins.)	Digestibility (%) Without enzyme	Digestibility (%) With enzyme	Nitrogen solubility (%) in aq. Na_2SO_3
–	34.5a	83.5a	87.0a
5	15.9b	85.5b	60.5b
15	13.7b	87.2b	44.2c
30	13.0b	85.2b	30.6c
45	13.8b	83.2a	28.5b
60	12.0c	80.0c	22.8c

* Dry matter basis. – Means of the same column followed by different letters differ significantly (P≤0.05) according to Duncan's Multiple Range Test. – All values of the table represent average of three replicates.

References

1. A. Uzzan, Chem. Industry, *The French Oils and Fats Industry, A Success Story* (1989), pp. 623-29.
2. F. X. Aherne and J. J. Kennelly, *Oilseed Meals for Livestock Feeding*, In: *Recent Advances* in *Animal Nutrition*. W. Hereresign (Ed.) (Butterworth, London, 1983), Ist ed., pp. 3-89.
3. A. H. K. Niazi, T. Kausar, F. H. Shah and W. Ishaque, Pak. j. sci. ind. res., **34,** 321 (1991).
4. H. M. Bau, Dj. Mohtadi-Nia, L. Majean and G. Derby, J. Am. Oil Chem. Soc., **60,** 1141 (1983).
5. M. C. S. Shastry and N. Subramanian, *ibid.*, **62,** 1131 (1985).
6. F. W. Sosulski and A. Bakal, J. Inst. Technol. Aliment, **2,** 28 (1969).
7. *Official Methods of Analysis* (AOAC, Washington D. C., 1980), 13th ed.
8. S. Gheyasuddin, C. M. Carter and K. F. Mattil, Fd. Technol., **24,** 242 (1970).
9. S. Mandal, S. G. Majumidar and C. R. Maity, J. Am. Oil Chem. Soc., **62,** 1124 (1985).
10. E. L. Wheeler and R. E. Ferrel, Cereal Chem., **48,** 312 (1971).
11. R. G. D. Steele, *Principles and Procedures of Statistics*, (McGraw Hill, Toronto, London, 1965), pp.345.
12. V. V. Klynchkin, P.P. Demchenko and V. N. Brik, J. Rio Grane Val. Hortic. Soc., **34,** 29 (1980).
13. F. S. Taha, A. Mehassen, A. S. El-Nockrashy and Z. E. Shoeb, J. Sci. Fd. Agric., **32,** 166 (1981).
14. A. K. Smith, A. Altschul (Ed). *Processed Plant Protein Foodstuffs* (Academic Press, New York).
15. T. Hortigome and M. Kendatsu, Agri. Biol. Chem., **32,** 1093 (1968).
16. M. C. S. Sastry, Ph.D. Thesis, Interactions with Sunflower Proteins, Univ. of Mysore, Mysore, India, (1984).
17. A. A. Betschart, R. Y. Fong and R. M. Saunder., J. Fd. Sci., **42,** 1088 (1977).