Technology Section

Pakistan J. Sci. Ind. Res., Vol. 31, No. 2, February 1988

DETOXIFICATION OF MUSTARD SEED CAKE

Elimination of Toxic and Antinutritive Factors from Mustard Seed Cake

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(Received July 15, 1987; revised February 9, 1988)

Optimum conditions for elimination of toxic antinutritive factors, i.e., allylisothiocyanate and phytic acid, from mustard seed cake have been investigated. Enzymatic detoxification followed by steeping in 4 % NaCl solution at pH 5 reduced allylisothiocyanate content to traces and eliminated 83.64 % of phytic acid respectively. Maximum nitrogen solubility profile in water, 10 % NaCl and 0.2 % NaOH was about 39.36 % and in vivo, digestibility (86.06 %) of dry matter of low phytate detoxified meal was observed at pH 5.

Key words: Glucosinolates, Phytic acid, Protein.

INTRODUCTION

Mustard/rape seed is an important source of vegetable oil, and is expected to decrease the shortage of 695 thousand tons of edible oil [1] in the country. Present in terest in the mustard/rape seed cake is (residue lift after oil extraction) as a new source of feed and food quality protein is a direct consequent of the fact that it has a well balanced amino acid profile [2]. However, association of toxic and antinutritive factors like glucosinolates, phytic acid and fibre [3-5] with mustard/rape seed protein, make it unfit for the non-ruminants and unpalatable for the ruminants. Until recently research was centred on the thyrotoxic and hepatotoxic effects of the hydrolysis products of glucosinolates [6]. Successful removal of the glucosinolates [7-9] together with the selection of low glucosinolate low erucic acid varieties have produced high quality protein [10] from mustard/rape seeds. Phytic acid present in seeds is not easily extractable, and several antinutritive properties, i.e. insoluble complex with several essential minerals and reduced protein utilization [11] have been ascribed to it.

The authors [12] have already suggested an economical procedure for complete removal of glucosinolate, resulting in a nutritionally better product. The object of the present studies were, a) to optimize conditions for simultaneous removal of glucosinolate and phytic acid, and b) to determine nitrogen solubility profile and in vivo digestibility of the low phytate-detoxified mustard seed meal.

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MATERIALS AND METHODS

Mustard seed (*Brassica juncea*) variety RL-18 was procured from Ayyub Agriculture Research Institute, Faisalabad.

Processing. The mustard seeds were pre-pressed twice in oil expeller (Handle Model 510, Japan), and oil contents further reduced to 2 % by soxhlet extraction with n-hexane for 20 hrs. The mustard seed meal (MSM), i.e. defatted cake, was dried at 60° ± 2° for 15 minutes and ground in a ball mill to 80 mesh size.

Detoxification of Mustard Seed Meal

1. *Elimination of Glucosinolate.* One kg of mustard seed meal was suspended in 5 litres of water and incubated at 55° ± 2° for 45 minutes for hydrolysis of glucosinolate by endogenous enzyme. Free allylisothiocyanate (AIT) was removed by steam stripping for 30 minutes [8]. The slurry was filtered and the residue dried at 80° ± 2°. The proteins were coagulated by steaming for 30 minutes and seperated by centrifugation at 3000 rpm for 30 minutes. The coagulant was added to the residue before drying [12].

2. *Elimination of glucosinolate and phytic acid.* The treated mustard seed meal slurry above [8] was obtained after steam stripping with sodium chloride so as to contain 4 % NaCl on ω/v basis. The effect of NaCl concentration was studied in the slurry by varying its concentration from 1 to 6 % at 30° and at pH 5 for one hour [13]. It was then filtered and the residue along with proteins were recovered from the filterate [12] and dried at 80° ± 2°. The dried meal was ground to 80 mesh size.

Nitrogen solubility profile. Two grams of the detoxified mustard seed meal 80 (mesh) was first extracted with

20 ml of water and then with 20 ml of 10 % NaCl, followed by 20 ml of 0.2 % NaOH in centrifuge tube with mechanical stirring for 2 hrs. at room temperature (30°). The slurries were centrifuged at 4,000 rpm. for 20 minutes and the soluble portion was decanted. Ten ml. of soluble portion was taken for nitrogen estimation by micro-Kjeldhal method [14]. The extracted nitrogen was expressed as the percentage of total meal nitrogen (N x 6.25). The experiments were conducted in triplicate.

Digestibility. In vivo digestibility of dry matter (including protein, ash, crude fibre and fat) of the detoxified meal was determined according to the procedure of Orskove & Macleod [15].

A dry Sahiwal cow weighing 350 kg was obtained from M/s. Packages Dairy Farms Ltd., Pakistan, and fistulated. The cow was given necessary treatment for one month and kept at maintenance ration. After one month, the samples of detoxified mustard seed meal were infused in the rumen as per standard technique in four replicates and taken out after 48 hrs. The samples were washed, dipped in alcohol for half an hour and washed again with distilled water until washings gave no colour for soluble materials. The washed samples were dried at 105° ± 2° to a constant weight on cooling in a dessicator containing calcium chloride. The co-efficient of digestibility was calculated as follows:

$$\text{Digestibility} = \frac{\text{Weight of sample infused} - \text{weight of sample left}}{\text{Weight of sample infused}} \times 100$$

Proximate analysis. Moisture, ash, crude fibre, protein and oil contents of the samples were determined by A.O.A.C. methods [16] whereas allylisothiocyanate and phytic acid in the untreated and treated mustard seed meal were estimated by the method of Wetter [17] and Wheeler [18], respectively.

RESULTS AND DISCUSSION

The proximate composition of mustard seeds (RL-18) is reported in Table 1. The moisture, protein, fat, ash, crude fibre, allylisothiocyanate and phytic acid were found to be 5.42, 27.24, 35.22, 4.40, 6.71, 0.85 and 1.84 %, respectively. The values obtained coincide with the findings of Shah *et al.* [19], with little variation in the amount of protein, oil, allylisothiocyanate which might have been due to the effect of fertilizer, soil composition, temperature and changes in the genetic characters of RL-18 seeds. The comparative values of composition of mustard seed cake and meal (MSM) obtained after prepressing of seeds. Solvent extraction (Table 1) showed that the increase in protein content to 37.75 % and 43.00 % in mustard seed cake and meal was mainly due to the amount of oil extracted. All other constituents also showed an increase on oil extraction.

According to the modified procedure of Shah *et al.* [12] for enzymatic hydrolysis of glucosinolate to allyisothiocyanate (AIT), which was eliminated by steam stripping. The AIT content of the detoxified mustard seed meal (DMSM) was reduced to non-detectable level. Detoxification resulted in a decrease in protein from 43.0 to 39.25 % but crude fibre and phytic acid contents increased from 10.7 to 12.95 % and 2.75 to 3.21 % respectively (Tables 1 and 2). The increase might be attributed to the removal of soluble fractions during treatment. The effect of NaCl concentration on dissolution of phytic acid and protein content of the meal is shown in Fig. 1. Maximum phytic acid (83.64 %) was extracted at 4 % NaCl concentration. The protein contents corresponding to the mini-

Table 1. Proximate composition* of mustard seeds, mustard seed cake and mustard seed meal.

Constituents analysed (%)	Mustard seeds	Mustard seed cake	Mustard seed meal
Moisture	5.42	8.31	4.33
Protein	27.24	37.75	43.00
Fat	35.22	16.15	2.05
Ash	4.40	5.64	6.81
Crude fibre	6.71	8.58	10.71
Allylisothiocyanate	0.85	1.08	1.22
Phytic acid	1.84	2.38	2.75
NFE**	23.74	28.41	33.46

*Dry matter basis; **Nitrogen free extract.

Table 2. Proximate composition of detoxified mustard seed meal (DMSM)

Constituents analysed (%)	Mustard seed meal	
	AIT** free (DMSM)	Low phytate (DMSM)
Moisture	5.28	6.15
Protein	39.25	38.42
Fat	2.35	2.44
Ash	6.60	7.91
Crude fibre	12.95	13.80
Allylisothiocyanate	Traces	Traces
Phytic acid	3.21	0.45
NFE	35.64	36.98

*Dry matter basis; **Allylisothiocyanate.

mum amount of phytic acid left was 68.4 % (protein basis). The findings are in agreement with the observations of Siy and Talbot [20] who observed that 4 % NaCl concentration was most suitable for phytic acid dissolution. The influence of pH on reduction of phytic acid in DMSM was investigated by using 4 % NaCl concentration at pH 4-8. The results showed that maximum dissolution of phytic acid occured at pH 5 (Table 3). This is in accordance with the observation of Johnson *et al.* [21] according to which phytates present in oilseeds have maximum solubility at pH 5, being more suitable because of comparatively lesser protein losses.

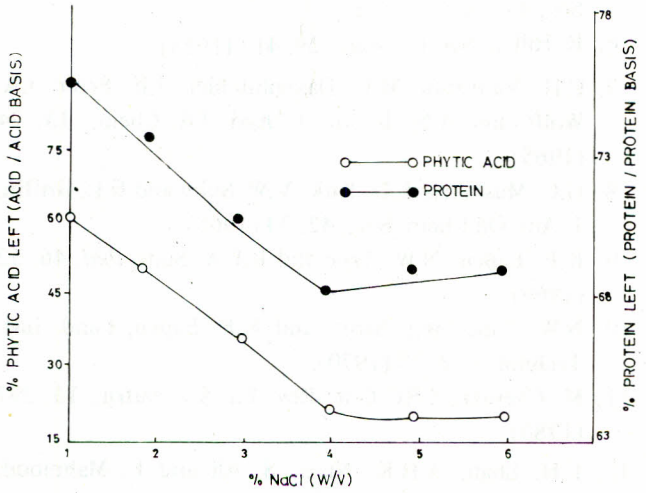

Fig. 1. Effect of NaCl concentration on phytic acid and protein contents of detoxified mustard seed meal.

The nitrogenous substances soluble in water and in NaCl are the protein fractions most easily assimilated by non-ruminants. Keeping this in view, nitrogen solubility profile of mustard protein in untreated MSM and DMSM was determined (Table 4). Maximum protein solubility was found in 0.2 % NaOH followed by water and 10 % NaCl in both cases. The decrease in solubility of DMSM might be due to losses of water soluble protein and partial denaturation by steaming during detoxification of MSM followed by drying at 80° ± 2°. This is in accordance with the observation of Rutkowski [23]. The solubility profile of low phytate-DMSM protein obtained after steeping the DMSM in 4 % NaCl solution at pH 4 to 8 Fig 2. The solubility profile in water, 10 % NaCl and 0.2 % NaOH at pH 4 to 8 varied from 7.18 % to 9.84 %, 5.12 % to 8.61 % and 13.32 % to 22.96 % respectively. The overall maximum

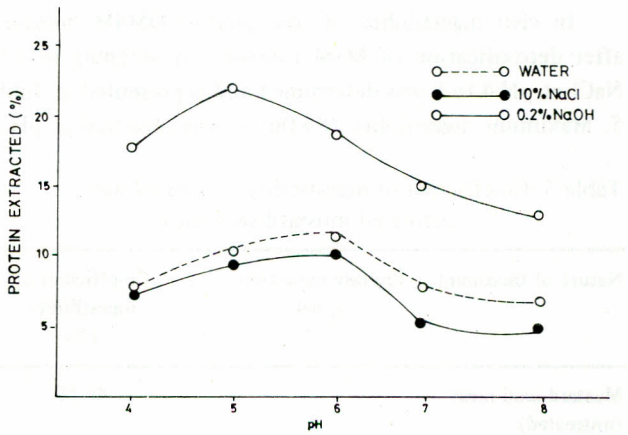

Fig. 2 Solubility profile of low phytate-detoxified mustard seed meal extracted with 4 % NaCl at pH 4-8.

Table 3. Effect on pH on reduction of phytic acid and composition* of detoxified mustard seed meal (DMSM) by steeping in 4 % NaCl.

pH	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	AIT (%)	Phytic acid (%)	Recovery (%) W/W basis
–(DMSM)	5.28	39.25	2.35	6.60	12.95	Traces	3.21	80.15
4	5.04	37.63	2.52	8.20	13.91	Traces	0.61	75.51
5	6.15	38.40	2.44	8.35	13.80	Traces	0.45	76.60
6	5.83	37.51	2.33	9.35	13.35	Traces	0.74	81.25
7	4.83	35.91	2.26	10.17	12.84	Traces	0.78	83.30
8	7.05	35.24	2.06	11.46	12.75	Traces	0.75	84.04

*Dry matter basis.

Table 4. Solubility profile of mustard protein.

Nitrogen soluble compounds	Mustard seed meal (untreated)	Detoxified mustard seed meal (DMSM)
Water	15.80	7.42
10 % NaCl	12.41	6.63
0.2 % NaOH	18.69	16.07
Insoluble**	53.10	69.88

*Dry matter basis; **By difference.

solubility (39.36 %) in all the solvents i.e. 8.61 %, 7.79 % and 22.96 % in water, 10 % NaCl and 0.2 % NaOH respectively, was found in low phytate-DMSM obtained after steeping at pH 5 and minimum (25.60 %) pH 8. The decrease in the solubility of protein at pH 4, 6, 7 and 8 seems to be due to the higher losses of soluble protein fractions during AIT and phytic acid removal from DMSM in this range of pH. Similar observations have been reported by Rutkowski and Korolczuk [24] about the extraction of proteins from rapeseed meal at different pH.

In vivo digestibility of low phytate-DMSM obtained after detoxification of MSM followed by steeping in 4 % NaCl at pH 4 to 8 was determined and is presented in Table 5. Maximum digestibility (86.06 %) was observed at pH 5

Table 5. Co-efficient of digestibility of treated and untreated mustard seed meal.

Nature of treatment	Phytate reduction at pH	Co-efficient of digestibility (%)
Mustard seed meal (untreated)	–	66.60
Detoxified mustard seed meal (DMSM)	–	75.51
Low phytate detoxified mustard seed meal	4	59.84
" " "	5	86.06
" " "	6	80.74
" " "	7	79.20
" " "	8	76.86

and minimum at pH 4 (59.84 %). Increase in pH from 5 to 8 showed decrease in digestibility from 86.06 % to 76.8 %. Lower digestibility of low phytate-DMSM at pH 4 and 8 might be due to partial denaturation of protein in the meal and formation of insoluble complexes of protein and minerals with phytic acid resulting in their reduced absorption [23,24]. From the present observations it is concluded that low phytate detoxified mustard seed meal containing 38.40 % protein and showing a total nitrogen solubility profile of 39.36 % (N/N basis) in water, 10 % NaCl and 0.2 % NaOH and in vivo digestibility of 86.06 % indicate its suitability for supplementation in the feed of non-ruminants and ruminants.

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