Pakistan J. Sci. Ind. Res., Vol. 20, Nos. 4-5, August-October 1977

DETOXIFICATION OF MUSTARD SEED CAKE

F. H. Shah, A. H. K. Niazi, Shaukat Ali and Ijaz Mahmood
PCSIR Laboratories, Lahore-16.

(Received May 15, 1976)

Abstract. Pakistan is the third largest producer of rape and mustard seeds in the world. The cake of the seeds after the extraction of the oils is used for incorporation into animal feed, with the rest being used as a manur or is exoprted. The cake containg 45% crude protein is not being utilized properly. The present study concerns itself with the detoxification of the mustard seed cake so that it is properly utilized.

Pakistan is among the major producers of rape and mustard seeds, being the third in the world.[1] The annual production of these seeds in Pakistan was 401,000 metric tons in 1970-71[2] and is likely to increase further.

The seeds are mainly used for extraction of the edible oil and an insignificant amount of the cake is used for incorporation into animal feed; the rest is either used as a manure or is exported. Thus the cake which contains upto 45% crude protein and can be incorporated into animal feed is not utilized properly within the country. The reasons for this wastage is the presence of glucosinolates in the cake which render it toxic for non-ruminants and unpalatable for the ruminants.

Georing *et. al.*[3] and Miller *et. al.*[4] studied amino acid contents of the cake and reported that the proteins were of high biological value. Detoxification of the oil seeds and the cake was carried out by various workers.[5-15] The present studies deal with the detoxification of cakes of Brassica seeds, especially *Brassica juncea* seeds.

Materials and Methods

Screw pressed oil-seed cakes of "Poorbi Raya", RL-18. local "sarson", and S-9 supplied by the Panjab Agriculture Research Station, Faisalabad, were extracted with haxane and detoxified by various methods.

Moisture Content. 2-3 g. of the sample were kept at 100° in an oven for 20 hr.

Protein. The nitrogen present in the sample was estimated by micro-Kjeldahl method using an SeO_2 : $CuSO_4$: K_2SO_4 (0.02 : 1 : 9) mixture.[16] A factor of 6.25 was used for conversion of nitrogen into protein contents.

Ash. 2-3 g. (D.M.) of the sample was incinerated at low flame and then kept in a muffled furnace for 5-6 hr at 450-500°.[17]

Crude Fibre. The crude fibre was estimated by Hemberg acid-alkali method.[19] Ash was determined by direct incineration method and finally by keeping the sample in a muffled furnace at a temperature 500-550° for 4 hr.

Fat. The fat present in the samples was extracted in a Soxhlet extractor[18] using *n*-haxane as a solvent.

Protein Efficiency Ratio (*PER*). *Iso*-nitrogenous diets, containing 10% protein were prepared from detoxified protein samples and semi-synthetic proteinous mixture. The control diet derived all its protein from casein. PER was determined by the method of Campbell[20] in four weeks' experiments.

Net Protein Utilization (*NPU*). The net protein utilization of the diets was determined according to the method of Miller and Bender.[21]

Volatile Isothiocyanate. Volatile isothiocyanate was estimated by the method of Wetter.[22]

The detoxification was carried out by the following methods. [5, 8, 11, 12, 23]

Enzymic Treatment. 1 kg. of the cake was suspended in 3 l. of water and incubated at 55 ± 0.5° for 45 min. for enzymic hydrolysis of the glucosilate. The free *iso*-thiocyanate was removed by steam distillation and the meal dried at 80 ± 5° in an oven or on a roller drum drier.

A portion of the suspension was fllter-pressed to separate the cake from excess of water. The filtrate was subjected to the following treatments :

(*i*) Dried at 100° in a steam jacketed pan,

(*ii*) Spray dried in a Niro spray drier (Copenhagen, Denmark). The flow rate of the drying material was adjusted to maintain the outlet temperature between 62-65°,

(*iii*) Steamed for 30 min. to coagulate the protein present. The coagulated proteins were separated by centrifugation at 3000 rpm for 30 min. and dried at 80±0.5°.

Aqueous Extraction. (*i*) The cake was soaked in five times its weight of water and kept at room temperature for 18 hr.

(*ii*) Eapen *et. al.* method. *Metallic ion Treatment.* 25 g. of the meal was soaked in 70 ml. of water containing 0.6 of $FeSO_4$. $7H_2O$ and autoclaved for 30 min. Allyl *iso*-thiocyanate formed during this treatment was extracted with chloroform.

Extraction with acetone. (*i*) 10 g. of the cake was blended with 5 times its volume of 80% acetone for 2 min and filtered.

(*ii*) Double extraction with 80% acetone was also carried out.

Toxicity Tests. The toxicity of various enzymic treated meals was tested by feeding it to rats for 105 days. All the diets were isonitrogenous and prepared according to the method of Ali[25] and Folly.[26] The rats were weighed after every 21 days.

Biological Detoxification. Locally isolated strains[24] of *Aspergillus niger*, *Aspergillus flavus* and *Bacillus subtilis* were employed for detoxification of the mustard seed cake.

Preparation of the Inoculum. 75 ml. Sauton medium[14] was transferred into 250 ml. Earlenmayer flask and sterilized at 110° for 15 min. 5 ml. suspension of 2 days old culture of bacteria, and 5 ml. suspension of 5 days old culture of *Aspergillus niger* and *Aspergillus flavus* containing 238,300 and 192,500 spores respectively was transferred asceptically to the respective flasks. The culture medium was incubated on a rotary agitator at 30±0.5° for 48 hr.

Detoxification of Mustard Seed Cake. The flasks containing 36 g. of mustard seed cake, 30 ml. culture medium and 120 ml. tap water were incubated at 30±0.5° for 48 hr with slow agitation. The fermented mustard seed cake was centrifuged (RPM=3000, time=45 min.) and the residue was oven dried at 60°. The dried cake was analyzed for its chemical composition.

Hydrolysis of Sinigrin. Sauton medium containing 0.07% sinigrin was inoculated with a loop of 5 days old culture of *A. niger*, *A. flavus* or 2 days old culture *B. subtilis* var. *niger*. The hydrolytic breakdown of sinigrin was studied during slow agitation at 30±0.5° for 48 hr.

Results and Discussions

Detoxification of Cakes. Table 1 shows that single extraction of RL-18 Poorbi Raya, S-9 and local sarson seed cakes with 80% acetone resulted in upto 37% decrease in allyl *iso*-thiocynate content. The maximum decrease, *i.e.* 37%, being in local sarson seed cake. Double extraction of the seed cakes with 80% acetone, resulted in upto 40.7% decrease in the allyl *iso*-thiocyanate content. The maximum detoxification was observed in case of local sarson.

The seed cakes detoxified by enzymic or ferrous sulphate treatment, aqueous extraction or extraction with 80% acetone followed by ferrous sulphate treatment contained traces of allyl *iso*-thiocyanate. It is evident from these results that maximum detoxification of the seed cakes can be easily accomplished by enzymic or aqueous treatment.

Biological Studies on the Cakes : The effect of different diets on the growth of rats is shown in Table 2. The growth rate of rats fed on control

TABLL 1. EFFECT OF DETOXIFICATION ON ALLYL *Iso*-THIOCYANATE CONTENT OF MUSTARD AND RAPE SEED CAKES

Treatment	Allyl *iso*-thiocyanate %			
	RL-18 (*B. juncea*)	Poorbi Raya (*B. juncea*)	S-9 (*B. juncea*)	Local Sarson (*B. comperstris*)
1. Nil	0.87	0.94	0.60	0.81
2. Single extraction with 80% acetone	0.63	0.71	0.39	0.51
3. Double extraction with 80% acetone	0.57	0.63	0.36	0.48
4. Enzymic treatment	Traces	Traces	Traces	Traces
5. Aqueous extraction	,,	,,	,,	,,
6. Ferrous sulphate treatment	,,	,,	,,	,,
7. Single Acetone extraction and ferrous sulphate treatment	,,	,,	,,	,,

TABLE 2. EFFECT OF INCORPORATION OF DETOXIFIED MUSTRAD SEED CAKE ON GROWTH AND NET PROTEIN UTILIZATION

Diet No.	Source of protein	Treatment	Increase in weight (g) during 10 days						Net protein Utilization (NPU%)
			0	2	4	6	8	10	
Control	Nil	Nil	150	142	137	135	132	138	
1	Mustard seed cake	Nil	150	140	135	134	131	127	45.80
2	Mustard seed cake	Aqueous extraction	150	178	178	184	194	203	57.40
3	Mustard seed cake	Enzymic treatment	150	175	180	184	192	198	60.07

diet (non-proteinous diet) and the diet containing mustard seed cake as such (un-detoxified), were somewhat identical. This indicated that the presence of toxic factor in the cake affected the nutritive value of protein in diet-1. The rats fed on enzymic treated mustard seed cake (diet-3) showed an increase in the body weight upto 49 grams over a period of 10 days. diet-2 containing aqueous extracted mustared seed cake also showed an increase in the body weight upto 53.0 grams during the same period.

Table 2 shows that NPU of diet-1, was 45.8% whereas that of diets 2 and 3 was 57.40 and 60.07% respectively. The increase in NPU appears to be due to the removal of toxic factors. Thus NPU of mustard seed cake was improved by enzymic treatment and aqueous extraction.

The growth rate of albino rats fed on diets 1-3, for 40 days is shown in Fig. 1. It is evident that growth was poor when the rats were fed on a diet containing mustard seed meal as such (diet 1). 25% of the rats died during 40 days feeding period.

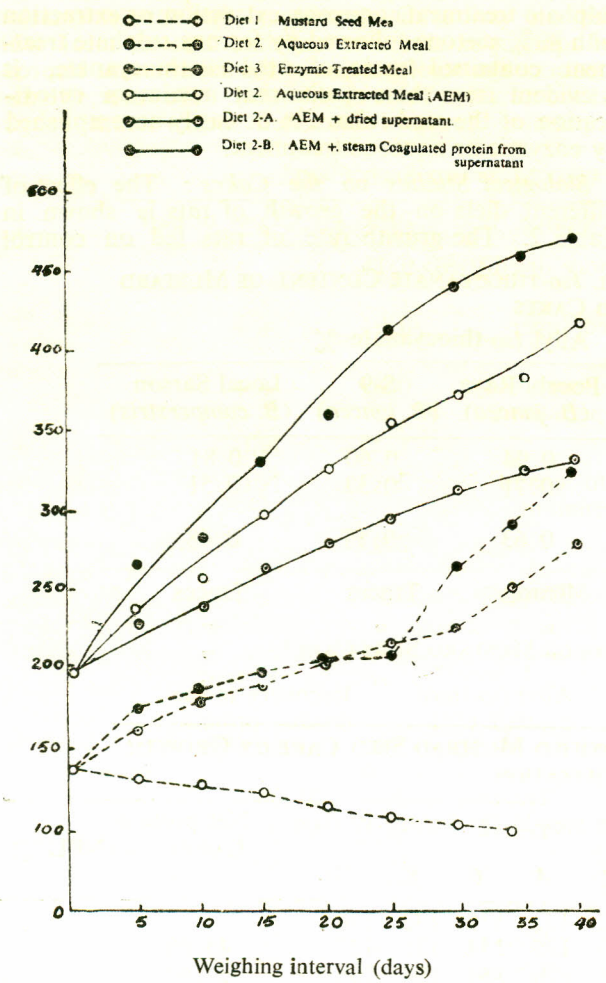

Fig. 1. The effect of supplemation of the detoxified cake residue with supernatants on the growth rate of rats.

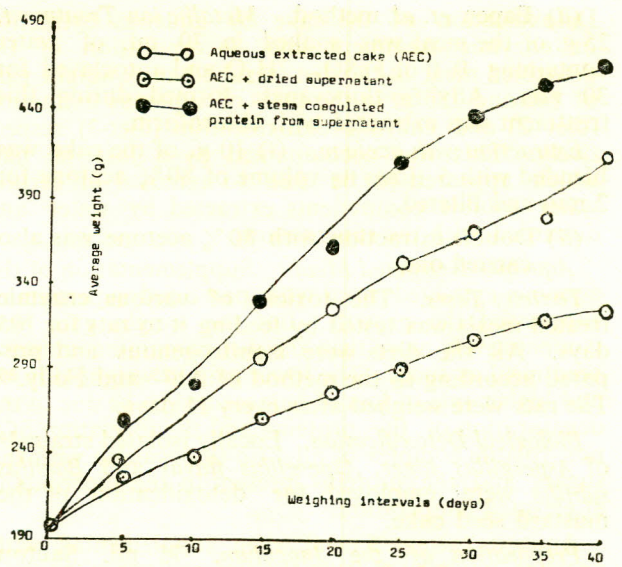

Fig. 2. Effect of supplementation of aqeous extracted mustard seed cake with supernants, on growth rate of rats.

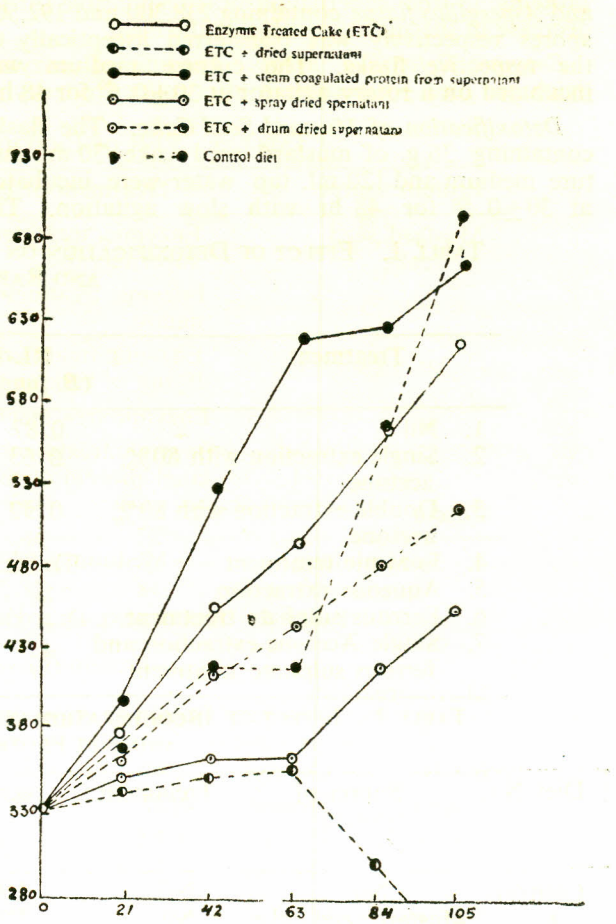

Fig. 3. Effect of the supplementation of the detoxified cake residue on the growth rate of rats.

Detoxification of the cake by aqueous extraction or enzymic treatment and incorporation of the detoxified meal in the diet resulted in a weight gain of 140 and 185 g. in the case of aqueous extraction (diet 2) and enzymic detoxification (diet-3) respectively. This was accompanied by a loss of hair which appeared to be due to removal of certain water soluble components extracted by water and discarded with the supernatant.

Aqueous Extracted Meal. Supplementation of the aqueous extracted meal with pan dried supernatant, suppressed the growth. But the weight gain was comparatively more than the diet containing mustard seed meal as such. The lower gain in weight (Fig. 2) and higher rate of loss of hair seemed to be due to the denaturation of the proteins and the formation of a non-volatile complex of allyl *iso*-thiocyanate with the sugars and the proteins present in the supernatant at elevated temperature.

Aqueous extracted meal when supplemented with proteins coagulated from the supernatant by passing steam showed an increase of 265 g. in the body weight of rats (Fig. 2). This improvement in the quality of the meal showed that loss of water soluble proteins in the supernatant was responsible for the decrease in the nutritive value of the meal.

Enzymic Detoxified Meal. Feeding of enzymic detoxified meal+supernatant after roller drum drying resulted in a weight gain of 193 g. and maximum loss of body hair.

Enzymic detoxified meal when supplemented with pan dried/spray dried supernatants showed a comparatively lesser weight gain (Fig. 3) and more loss of hair. Death of all the rats fed on the diet containing pan dried supernatant after 84 days, indicated that free allyl *iso*-thiocyanate, formed during enzymic detoxification, formed highly toxic substances, during pan drying, with the carbohydrates and protein present in the supernatant.

PER Determination. PER of the casein based diet and the experimental diets are given in Table 3. It is evident that the supplementation of the enzymic detoxified meal with steam coagulated proteins of the supernatant improved the PER of the meal. Decrease in PER of the diets containing the roller-drum dried, enzymic detoxified meal was due to the presence of toxic and denatured proteins present in the supernatant which separated out from the residue and was mixed with it after pan or spray drying.

Growth rate (Fig. 2-3) and loss of hair of rats fed on various diets confirmed that supplementation of aqueous extracted or enzymic treated meal with steam coagulated proteins of the supernatant improved the nutritive value of the meal. However, the diet containing enzymic detoxified meal+steam coagu-

TABLE 3. PER OF ENZYMIC TREATED MUSTARD SEED CAKE

Protein source	Treatment of Protein Source	PER	Loss of hair
Mustard seed cake	Enzymic treatment+spray dried supernatant	0.15	+
,,	Enzymic treatment+dried supernatant	0.94	++
,,	Enzymic treatment and drum drying of residue and supernatant	0.89	+++
,,	Enzymic treatment	1.43	Nil
,,	Enzymic treatment+steam coagulated protein of the supernatant	2.17	Nil
Casein	Nil	2.43	

+Minimum ++Medium +++Maximum

TABLE 4. BIOLOGICAL DETOXIFICATION OF MUSTARD SEED CAKE*

Micro-organism		Protein (g)		Allyl *iso*-thiocyanate (%)	Recovery in the residue protein/protein (%)
		Residue	Supernatant		
Bacilus subtilis	Control	3.66	3.22	Traces	53.36
	Treated	4.27	3.28	Traces	62.25
Aspergillus niger	Control	3.85	3.11	Traces	54.80
	Treated	4.39	3.18	Traces	63.98
Aspergillus flauvs	Control	3.52	3.06	Traces	53.53
	Treated	4.06	3.18	Traces	61.67

*After 48 hours.

lated proteins of the supernatant showed comparatively better observed in case of the former.

Biological Detoxification. Biologicol detoxification of mustard seed cake by locally isolated strains of *Aspergillus niger*, *Aspergillus flavus* and *Bacillus subtilis* var. *niger* showing allyl *iso*-thiocyanate was completely eliminated by all micro-organisms after 48 hr of incubation (Table 4). An increase in the protein content of the fermented meal from 8.14 to 9.71% was also observed (Table 4). The maximum increase (9.71%) in the case of *Aspergillus niger* and the minimum (8.14%) in the case of *Bacillus subtilis* var *niger* was observed after 48 hours.

Aspergillus niger, *Aspergillus flavus* and *Bacillus subtilis* var *niger* were propagated on Sauton medium containing pure sinigrin as the sole source of carbon. Only *Aspergillus flavus* utilized sinigrin whereas the other two micro-organisms could not grow.

References

1. Commonwealth Econ. Commit., 1936-64, Vegetable Oil and Oil Seeds, H. M. Stationery Office, London.
2. All Pakistan Final Estimates of Crops, released by the Ministry of Food, Govern. Pakistan.
3. K. J. Goering and W. A. Curran, J. Nut., **72**, 210 (1960).
4. R. W. Miller, C. H. VanEtten, C. M. C. Grew and I. A. Wolf, J. Agr. Food Chem. **10**, 426-30, (1962).
5. C. H. VanEtten, M. E. Daxenbichler, J. E. Peter, I. A. Wolf and A. N. Booth, J. Agr. Food Chem., **13**, 24-26, (1965).
6. Kirk, L. D., G. C. Mustakas and M. L. Griffins Jr., J. A. O. C. S., **43**, 550-555, (1966).
7. Masood, Younus and F. H. Shah, Pakistan J. Sci. Ind. Res., **9**, 2 (1966).
8. G. C. Mustakas, L. D. Kirk, V. E. Sohns, and B. L. Griffins, J. A. O. C. S., **42**, 33-37, (1965).
9. L. D. Kirk, L. T. Black and G. C. Mustakas, J. A. O. C. S., **41**, 599-602, (1964).
10. J. E. MacGhee, L. D. Kirk, and G. C. Mustakas, J. A. O. C. S., **41**, 359-62, (1964).
11. C. G. Youngs and A. S. Perlin, Canad. J. Chem. **45**, 1801-1804 (1967).
12. K. E. Eapen N. W. Tape, and R. P. A. Sims, J. A. O. C. S., **46**, 52 (1969).
13. K. E. Eapen N. W. Tape and R. P. S. Sims J. A. O. C. S., **45**, 194 (1968).
14. T. Staron, Proc. Intern. Conf. Sci. Technol. and Marketing of Rape-seed and Rape-seed Products., p. 321, published by Rape-seed Assoc. Canada, Sep. (1970).
15. R. S. Bhatty and F. W. Susulski, J. A. O. C. S. **49**, 346-350 (1972).
16. AOAC 38,009, page 643, 10th edition 1960 (Mod).
17. AOAC 13,006, p. 158, 10th ed. (1960).
18. AOAC 22,003, p. 287, 10th ed. (1960).
19. AOAC 22,038, p. 288, 10th ed. (1960).
20. J. A. Campbell, *Methodology of Protein Evaluation.* Nut. Doc. R. 10/add. 27, WHO, FAO, UNICEF, PAG, New York, (1961).
21. D. S. Miller, and A. E. Bender, Brit. J. Nut., **9**, 382 (1955).
22. L. R. Wetter, Canad. J. Biochem. and Phys., **33**, 980 (1955).
23. G. C. Mustakas, L. D. Kirk, E. L. Griffins, J. A. O. C. S., **39**, 372 (1962).
24. A. Majid, Shaukat Ali and F. H. Shah, Pakistan J. Sci. Ind. Res., (Accepted for publication).
25. S. M., Ali, M. Hanif and A. S. Alvi, Pakistan J Sci. Ind. Res., **15**, 4, 153, Oct., (1961).
26. S. J., Folley, K. M., Nenry, & S. K. Kon. Brit. J. Nut. **1**, 39 (1947).