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PRODUCTION OF PROTEIN RICH MEAL FROM MUSTARD SEED KERNELS

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Optimum conditions for the detoxification of mustard meal (defatted kernel) were studied. pH 3.0 and 7 were found to be optimum for the detoxification of meal by enzymic treatment and aqueous extraction respectively. Comparatively better NPU (67.2%) and PER (2.35%) values of enzymic treated meal suggest its suitability for food formulation.

INTRODUCTION

Animal proteins which are known to contain balanced proportion of essential amino acids constitute only a fraction of the Pakistani diet. Nearly all their food protein is derived from cereals and legumes which are deficient in certain essential amino acids. This imbalance can be rectified through supplementation with such sources as are endowed with balanced amino acid profile. Dried milk, casein, fish meal and what are by far the most abundant and economical oilseed meals like those of rape, mustard and cotton, may be added to legumes and cereals to give nutritionally wholesome diets. However, this course cannot be adopted because of the toxic nature of the meals in raw form. Thus whereas the annual production of mustard and rapeseeds in Pakistan is estimated to be 275,000 tons per year and is likely to increase in view of the shortage of edible oil in the country only a small proportion of the meal is being utilized as poultry or animal feed, because the nutritional quality of the meal is impaired due to the presence of glucosinolates, high fibre [2] content and phytic acid [3].

The object of the present studies was to develop a process which would completely remove the toxic factor [1], allylisothiocyanate, from the defatted mustard kernels. Although many procedures [2–10] have been reported in literature to remove the toxic factor from mustard seed meal and workers like Eapon [11] and Tape *et.al.* [12] have tried to produce a white bland defatted rape seed flour free from both toxic factor and seed coat so as to extend its use in food products, yet the results obtained have remained unsatisfactory. The method suggested in the present communication is economical in energy consumption, gives nutritionally a better product and is free from toxic substances.

MATERIALS AND METHODS

Mustard seed varieties of RL-18, S-9, *local sarson* and *poorbi raya* (Brassica juncea) supplied by the Punjab Agriculture Research Institute, Faisalabad, were used in the present investigations.

Dehulling of Mustard Seeds [13]. The seeds were steamed for 15 min. dried at 80-90^{o} for an hour and passed through rollers to crack the hulls. The crushed seeds were allowed to fall down from a hole in the centre of a drum (length: 90 cm, diameter: 50 cm; open on both sides) and were subjected to air draught at right angle. The hulls being lighter were blown away to a further distance and the kernels settled in the form of a heap due to difference in their particle size.

Mustard Meal. 2 kg of mustard seed kernels (16.mesh) were refluxed in a solvent extractor for 20 hr. with n-hexane to reduce the oil content to minimum (2%) and dried at 50 ± 5^{o}.

pH Treatment. Mustard meals (defatted kernels) of RL-18, S-9, *local sarson* and *poorbi raya* were detoxified with endo and exoenzymes [6,11] i.e. myrosinase at pH 3 to 12.

1. 100 g of mustard meal were mixed with 25 g of myrosinase powder and 425 ml of water having different pH, i.e. from 3-12. The slurry was incubated at 55 ± 2^{o} for 15 min. for enzymic hydrolysis of glucosinolate. Steam stripping was carried for half an hour, the slurry filtered and residue dried at 80 ± 5^{o}.
2. 100 g of mustard meal were mixed with 500 ml of water having different pH range from 3 to 12. The slurry was stirred in a water bath at 60 ± 2^{o} for half an hour and filtered. The residue was again stirred and filtered in the same manner. The solid product obtained after second extraction [11] was dried at 80 ± 5^{o}. Mustard seed kernels treated and untreated were powdered to 22-mesh size and

analysed for protein, volatile allyl isothiocyanate, crude fibre, ash and moisture contents.

Moisture Contents. 2-3 g of the sample were kept at 100 ± 5° in an oven for 24 hr. [14] cooled in desiccator and weighed.

$$\text{moisture} = \frac{\text{Weight loss x 100}}{\text{Weight of sample}}$$

Protein. The nitrogen present in the sample was estimated by the micro-Kjeldhal method [15] using K_2SO_4: $CuSO_4$: SeO_2 (9:1:0.02) mixture. A factor of 6.25 was employed for conversion of nitrogen into protein.

Oil. The oil present in the sample was extracted in a Soxhlet extractor using n-hexane as a solvent [16]. The flask and the extract were kept in a water bath at 100 ± 5° until constant weight was attained.

$$\% \text{ oil} = \frac{\text{Weight of residue x 100}}{\text{weight of sample.}}$$

Crude Fibre. Crude fibre was estimated by the Hamberg acid alkali method [17].

Ash. 2-3 g of the sample were incinerated at low flame and then kept in a muffled furnace for 5-6 hr. at 450-550° [18].

Allyl isothiocyanate. Volatile allyl isothiocyanate was estimated by Wetter's method [19].

Net Protein Utilization (NPU). 22-mesh powder of RL-18 meal was aqueous extracted at pH 7 or treated with the enzyme at pH 3.0. It was incorporated into a semi-synthetic non-proteinous diet in such a way that experimental diets were isonitrogenous, i.e. contained nearly 10% protein. The Net Protein Utilization of the diet was determined according to the method of Miller *et. at.* [20].

Protein Efficiency Ratio (PER). Isonitrogenous diets, containing 10% protein, were prepared from aqueous extracted, enzymic treated mustard meal and semi-synthetic protein mixture. The enzymic treatment and aqu-

Table 1. Effect of Aqueous extraction and pH on the composition of Mustard meals*

Meal constituents	Meal as such	pH					
		3	6	7	8	9	12
Moisture (%)							
RL-18	5.9	9.2	7.3	7.8	7.8	5.8	2.9
S-9	6.3	8.9	7.0	8.0	8.8	6.6	4.7
Local *sarson*	5.9	9.2	7.3	7.8	7.8	5.8	2.8
Poorbi raya	7.8	9.8	7.1	9.3	7.2	7.7	4.5
Protein (%)							
RL-18	50.6	56.2	55.4	58.2	58.8	58.5	55.3
S-9	51.7	56.5	60.0	60.3	58.2	56.5	55.6
Local *Sarson*	50.6	56.2	55.4	58.2	58.2	58.5	55.3
Poorbi raya	51.4	55.8	54.9	56.7	52.3	60.3	54.5
Allyl isothiocyanate (%)							
RL-18	1.76	0.15	0.11	Traces	Traces	Traces	0.04
S-9	1.68	0.12	0.08	Traces	Traces	Traces	0.04
Local *sarson*	1.76	0.15	0.11	Traces	Traces	Traces	0.04
Poorbi raya	1.80	0.04	Traces	Traces	Traces	Traces	Traces
Recovery % (W/W Basis)							
RL-18	--	75.9	68.8	69.5	62.4	65.9	63.1
S-9	--	73.6	71.0	65.0	68.2	70.7	63.1
Local *sarson*	--	77.1	68.4	59.6	59.6	67.4	63.6
Poorbi raya	--	75.3	70.0	70.6	65.8	69.3	70.7

* On dry matter basis.

eous extraction of meal was carried at pH 3.0 and 7 respectively. The control diet derived all its proteins from casein. PER was determined by the method of Campbell [21]. The composition of the experimental diets is shown in Table 3.

RESULTS AND DISCUSSION

Aqueous extraction and enzymic treatment of the defatted mustard seed kernel were carried out at pH ranging from 3 to 12 in order to determine the pH at which removal of allyl isothiocyanate as well as the recovery of protein was optimum.

The effect of various pH adjustment (pH 3-12) during aqueous extraction of RL-18, S-9, local *sarson* and *poorbi raya* meals showed that allyl isothiocyanate was completely eliminated at pH 7-9 (Table 1). An increase in pH from 9 to 12 was not effective for complete detoxification except in *poorbi raya*. The detoxified product obtained at pH 9 had a slightly higher protein content in the case of RL-18 (58.5 %), *local sarson* (58.5 %) and *poorbi raya* (60.3 %) than at pH 7. The slurry obtained at pH 8 and 9 was mucilaginous and thus the process of recovery of meal was extremely slow. Detoxification at pH 7 gave slightly less recovery of the meal than at pH 9 but was less time-consuming and was preferred.

The effect of pH adjustment (pH 3-12) in the presence of additional myrosinase (enzymic treatment) on the toxic factor of RL-18, S-9, *local sarson* and *poorbi raya* meals detoxified in the presence and absence of additional myrosinase (i.e. enzymic treatment and aqueous extraction) showed that both treatments gave products with slight difference in protein content. Adjustment of pH in the presence of myrosinase was more effective in the elimination of toxic factor and gave comparatively higher recovery of meal at pH 3 (Table 2).

Table 2. Effect of enzymic treatment and pH on the composition of mustard meals.*

Meal constituents	Meal as such	pH								
		3.0	3.5	4.0	4.5	5.0	5.5	6.0	9.0	12.0
Moisture (%)										
RL-18	5.9	3.8	3.7	6.3	3.0	2.2	4.0	4.6	4.2	5.2
S-9	6.3	5.2	4.0	6.7	2.5	1.9	3.4	5.7	1.6	5.2
Local *sarson*	5.9	6.2	4.4	6.8	2.7	2.2	4.0	4.9	4.5	4.5
Poorbi raya	7.8	2.9	4.1	6.8	3.0	2.1	4.2	1.2	1.6	1.8
Protein (%)										
RL-18	50.6	53.6	53.8	54.2	51.4	51.0	56.1	53.1	53.7	54.4
S-9	51.7	60.8	56.2	56.4	56.9	54.4	59.9	56.7	57.4	60.4
Local *sarson*	50.6	59.0	56.4	56.7	59.0	55.6	58.9	56.0	58.9	58.0
Poorbi raya	51.4	55.4	56.2	57.7	54.2	55.6	59.1	54.8	55.8	56.8
Ally isothiocyanate (%)										
RL-18	1.76	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces	Traces
S-9	1.68	"	"	"	"	"	"	"	"	"
Local *arson*	1.76	"	"	"	"	"	"	"	"	"
Poorbi raya	1.80	"	"	"	"	"	"	"	"	"
Recovery % (W/W Basis)										
RL-18	–	68.8	64.4	64.0	64.5	65.6	66.6	62.3	62.9	66.5
S-9	–	68.1	66.6	64.3	64.7	61.3	66.2	66.8	66.1	65.9
Local *sarson*	–	70.3	64.8	64.4	66.8	63.6	63.6	68.9	67.5	66.6
Poorbi raya	–	74.2	62.5	65.5	66.8	68.6	66.7	71.1	73.7	76.3

*On dry matter basis

NPU and PER of Mustard Meal. The protein content, nitrogen retention, fecal nitrogen, NPU, PER and weight gain data of rats fed on standard casein diet and the experimental diets are shown in Table 4. The weight gain of rats fed on the diets containing casein, aqueous-extracted and enzymic-treated mustard meal was 82.0, 74.7 and 73.5 g. respectively after 10 days (Fig. 1). The diet containing aqueous-extracted mustard meal showed slightly better weight gain in rats than in the enzymic treated meal but lesser gain than the casein diet. The nitrogen intake, nitrogen retained and PER of casein diet and diet supplemented with enzymic treated mustard meal were almost equal. NPU of the casein diet was maximum, i.e. 75.0 % followed by the enzymic treated meal (67.2 %).

The improvement in the nutritional value of enzymic detoxified mustard meal may be largely attributed to the complete removal of the toxic factor. The lower NPU and PER values of aqueous extracted meal appear to be due to the presence of allyl isothiocyanate in the bound form or loss of some growth factor during extraction. Shah *et.al.* [10] have reported that presence of toxic factor in the

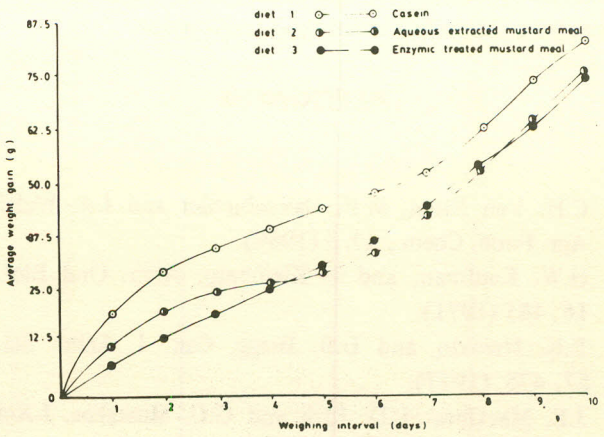

Fig. 1. Effect of incorporation of detoxified mustard meal on growth of rats.

Table 3: Composition of experimental diets

Ingredients	Casein (Diet 1)	Aqueous extracted RL-18, mustard meal (diet 2)	Enzymic treated RL-18, mustard meal (diet 3)	Non-protein
Mustard meal	–	18.8	19.4	–
Glucose	10.0	10.0	10.0	10.0
Vitamins	5.0	5.0	5.0	5.0
Minerals	5.0	5.0	5.0	5.0
Corn oil	8.0	11.2	9.0	8.0
Corn starch	58.9	49.0	51.6	70.0
Cellulose	–2.0	–	–	2.0
Casein	11.1	–	–	–
	100.0	100.0	100.0	100.0

Table 4: NPU and PER value of detoxified mustard meal of RL-18

Diet No.	Protein source	Protein %	N intake (g)	N retained (g)	Fecal nitrogen (g)	Weight gain (g)	NPU %	PER
1.	Casein	10.0	5.3	9.6	0.6	82.0	75.0	2.35
2.	Aqueous extracted mustard meal	9.6	6.0	9.5	1.0	74.7	64.7	2.10
3.	Enzymic treated mustard meal	9.8	5.2	9.2	1.0	73.5	67.2	2.25

untreated meal depressed the growth of rats. NPU and PER value of enzymic treated mustard meal indicated that mustard meal protein is of good quality. The results of this study suggest that mustard meal has a high potential as a protein source in human food formulation.

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