PREPARATION OF BLAND PROTEIN MEAL FROM MUSTARD SEED CAKE

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Attempts were made to convert an animal feed into palatable protein meal for human consumption. Allylisothiocyanate, a toxic substance, present in mustard seeds was liberated by enzymic hydrolysis of its glucoside and removed by steam distillation. The product thus obtained was free from the toxic material and contained 33.0% proteins on the fat-free basis. Crisp Pakoras prepared from the proteins were fairly acceptable and contained 16.5% proteins on fat-free basis. Net protein utilization value of the meal was 42%.

Introduction

Oriental mustard seed (*Brassica juncea*) grows abundantly in Pakistan (361,000 tons/year) and other parts of the world. It has high oil content (45%) and contains substantial amount of proteins (32.4%). In Western countries, it is used as a condiment while in Pakistan and India, oil from its seeds is used for frying purposes and the cake or the residue left after the expulsion of the oil is used as an animal feed. Mustard seed cake contains 27.6% proteins, (on fat-free basis) has a biting taste and is toxic to human beings. Oil contents of the cake vary from 10-12.5 percent.

Goering *et al.*[1] while studying the nutritional values of mustard meal reported that the meal obtained after enzymic hydrolysis of the seeds, was free from the toxic material. This process was patented by Goering[2] and later simplified by Mustakas *et al.*[3] The present work was undertaken with a view to study the utilization of mustard seed cake for human consumption.

Materials and Methods

Mustard seed cake was purchased from the local market. Four samples were mixed together to get a representative mixture and ground to 8-mesh size.

Analysis of Mustard Seeds and Cake.—Dry matter content of the mustard seeds, cake and meal was estimated by drying them at 98-100°C. for 5-6 hours. Ash content was determined by igniting the samples at 550°C. for 7 hours and the oil contents by extraction with petroleum ether (50°C.).

Meal (200 mg.) was suspended in 10 ml. of 10% trichloroacetic acid (TCA) solution and the TCA soluble nitrogenous matter was removed by centrifugation. The process was repeated twice to remove most of the soluble matter, and finally the residue was washed with water (10 ml.). The TCA soluble and insoluble portions will be referred in the text, as non-proteinous and proteinous fractions respectively.

Nitrogen was determined by a microkjeldahl method using K_2SO_4: $CuSO_4$: SeO_2(9:1:0.02) catalyst mixture and the protein calculated therefrom.[4] The results are represented in Table 1.

TABLE 1.—COMPOSITION OF MUSTARD PRODUCTS (%).

	Moisture	Oil	Ash	Proteinous nitrogen	Non-proteinous nitrogen
1.	Mustard seed 6.4	45.0	3.3	5.4	1.6
2.	Mustard cake 3.3	12.5	7.5	4.6	1.2
3.	Bland protein meal 8.5	18.2	9.0	5.5	1.1

Allylisothiocyanate was estimated by the method of Wetter.[5]

Germination of the Seeds.—Black mustard seeds (500 g.) were soaked in tap water for 24 hours. These were then spread on jute bags, in a germinating cabinet, at a temperature of 25±2°C. After 24 hours of germination, the seeds were mixed with a small quantity of water and made into a paste (dry matter 30%).

Preparation of the Meal.—Mustard cake (200 g.) were mixed with germinated seeds (150 g.) and pure sodium chloride (10 g.). The mixture was incubated at 56±2°C. in an incubator for 2 hours. The product was steam-distilled and cooked for 1½ hours. The moistened meal was then spread on trays which were kept overnight, in an oven at 60-70°C. The final product was then powdered to 60 mesh size and kept for further experiments.

The Effect of Heat on the Solubility of the Proteins.—The solubility of proteins was determined by taking mustard seed, mustard cake or the bland meal (2 g.) separately in 100 ml. of distilled water. This was stirred for 20 minutes and then centrifuged at 2000×g. for 15 minutes. The clear extract was filtered and the residue was extracted with two 25 ml. portions of distilled water for 20 minutes. These extracts were combined and the

total nitrogen extracted was determined. The residue after removal of the water-soluble materials, was extracted in the same way using three portions of 5% KCl solution. After this extraction, the residue was extracted for 20 minutes with 70% C_2H_5OH at 70°C. Finally, the residue was suspended in three successive portions of KOH solution and stirred for 20 minutes. The experiment was repeated with defatted material.

Determination of Net Protein Utilization.—Semi-synthetic isonitrogenous diet containing 10% proteins (on dry matter basis of the meal) was prepared by mixing the bland protein meal, Glaxo salt mixture and vitamin mixture.

The N.P.U. at 10% protein level was determined according to the method of Miller and Bender[6] using male albino rats, weighing 35-40 g. for a period of ten days.

Toxicity of the Protein Meal.—Feeding trials were conducted using albino rats, weighing 50-55 g. Four males and 4 females were used for feed treatment.

Preparation of Pakoras.—Bland mustard meal was mixed with gram flour, spices and water to form a thick paste. The mixture was transferred in small lots to a frying pan containing mustard oil and fried. The fried product "Pakoras" were tasted with and without tomato ketchup and found acceptable. The composition of Pakoras was: moisture 35%, oil 41% and protein 16.5 percent.

Results and Discussion

The compound responsible for the toxicity of the mustard seeds is sinigrin[7] which on hydrolysis yields allylisothiocyanate. The hydrolysis is brought about by an enzyme "myrosinase" which occurs naturally in the seeds. These enzymes are, however, destroyed during the extraction of oil, from the seeds, at an elevated temperature.

This loss was made up by the addition of germinated mustard seeds to a slurry of the cake. The active enzymes present in the germinated seeds liberated allylisothiocyanate which was finally removed by steam distillation. The optimum pH for the hydrolysis of sinigrin by the germinated seeds was determined by employing citrate buffer.[5] The amount of allylisothiocyanate liberated at various pH was determined as mentioned in the text. Maximum release of allyl-isothiocyanate occurred at pH 4 (Fig. 1).

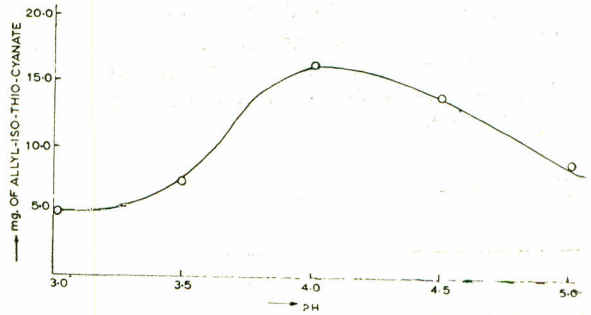

Fig. 1.—Showing effect of pH on the Liberation of Allyl-isothiocyanate.

Sandberg *et al.*[8] suggested that the myrosinase isolated from white mustard seeds was active over a wide range of hydrogen ion concentration. Ishimoto and Yamashina[9] reported pH optima of 5.0 to 5.2 for the enzymes isolated from three plant sources. The enzymes present in the black mustard seeds seems to have optimum activity at pH 4.

The amount of germinated seeds required for the complete hydrolysis of sinigrin is given in Fig. 2.

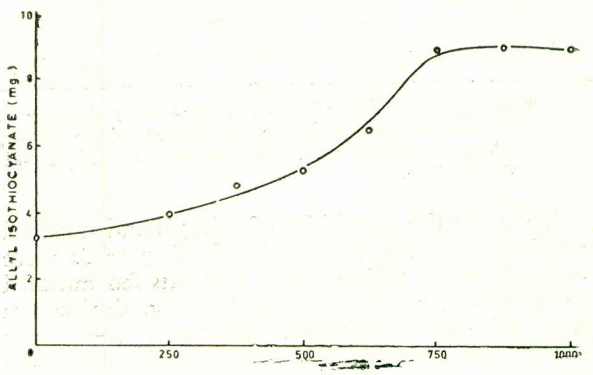

Fig. 2.—Showing liberation of allylisothiocyanate by germinated seeds.

22.5 g. (dry matter) of the germinated seeds were sufficient for the complete hydrolysis of the sinigrin present in 100 g. of the cake. A further increase in the amount of germinated seeds did not liberate any more allylisothiocyanate. Addition of NaCl to the slurry as suggested by Clapp *et al.*[10] for cabbage leaves, improved the taste of the product.

Mustard seeds contain all the essential amino-acids and have high contents of lysine, methionine and tryptophan.[1] Processing at elevated temperature, for the extraction of oil, seems to have adverse effect on the quality of the protein. Goer-

ing *et al.* suggested that the decrease in the nutritive value of the mustard meal could be either due to destruction of lysine or to the conversion of the protein to a more insoluble form. The experiments carried out on the solubility of the protein (Tables 2 and 3), show that the denaturation of the protein is one of the factors responsible for this decrease.

TABLE 2.—EFFECT OF HEAT ON THE SOLUBILITY OF PROTEINS (%).

	Water	5% KCl	70% C_2H_5CH	0.2% KCH
1. Mustard seed	3.0	3.7	2.2	2.4
2. Mustard cake	2.2	3.0	1.1	1.8
3. Bland protein meal	1.8	1.2	0.9	1.5

TABLE 3.—LUND-SANDSTROM FRACTION OF MUSTARD PRODUCTS. % OF TOTAL NITROGEN OF DEFATTED MATERIAL.*

Sample	I	II	III	IV	V
Mustard seed	15.71	18.14	3.43	8.57	54.15
Mustard cake	10.32	12.06	8.10	8.25	61.27
Bland protein meal.	8.48	8.64	5.16	6.52	71.20

* on dry matter basis; I. Cold Water-soluble fraction; II. Fraction soluble in 5% KCl; III. Fraction soluble in 70% Ethanol at 65-70°C.; IV. Fraction in cold 0.2% KOH; V. Residue.

To study the possibility of using bland mustard meal as a source of protein, feeding trials were carried out on albino rats. The rats fed mustard meal gained a weight of 25 to 32 g. during ten days of the experiment. The rats were very active and no other side-effects of the diet were noticed. These findings confirmed the results reported by Goering *et al.*[1] The net protein utilization value of the protein meal was 42.0 percent.

In order to determine whether any toxic factors were present in the bland protein meal, a feeding experiment was set up. This trial consisted of feeding rats a diet in which 50% of the proteins were from the bland protein meal. The gain in weight of the rats (Table 4) shows that the meal

TABLE 4.—TOXICITY TEST OF BLAND PROTEIN MEAL.

Sex	Total weight of the group at the start of the experiment. (g.)	Total weight after 5 weeks. (g.)
Male	215	365
Female	207	330

was not toxic. These results confirmed the findings of Goering *et al.*

The bland mustard meal was mixed with gram flour and spices and fried in mustard oil. The "Pakoras" thus prepared contained 2.3 g. each of protein and were very delicious.

From the results reported above, it is apparent that the bland protein meal can be safely used as a protein source for human beings.

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