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PREPARATION OF DIETARY FIBRE FROM MUSTARD SEED MEAL

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Investigations were carried out to reduce cellulose and hemicellulose from mustard seed meal by diferent methods to prepared dietary fibre. Reduction in hemicellulose was found to be 28.31% when detoxified mustard seed meal was autoclaved at 15 lbs/inch^2 for 30 mins. A significant reduction in cellulose and hemicellulose was also observed on treatment with sodium bicarbonate and sodium carbonate. A further reduction in these fibrous components was found on autoclaving the sodium bicarbonate or sodium carbonate treated meal. Reduction in cellulose contents was 26.45% after fermentation of detoxified meal with *Trichoderma viride* for 72 hrs. Protein digestibility of the meal was improved with the application of these treatments.

Key words: Mustard seed meal, Dietary fibre, Cellulose, Hemicellulose.

Introduction

Mustard seed meal contains 35-40% protein which has a well balanced amino acid profile [1]. Presence of relatively high concentration of basic sulphur containing amino acids [2], makes it a suitable complement to cereals and many legumes which are deficient in these amino acids. It can be utilized as a rich source of protein and dietary fibre for human consumption but the presence of anti nutritional factors and toxic substances such as allyl-isothiocyanate and phytic acid; and high amount of polysacharides limits its utilization as human food. Allyl-isothiocyanate and phytic acid have been successfully reduced in the meal and it has been evaluated for its protein efficiency by conducting nutritional experiments on albino rats [3-5]. However, the presence of fibrous matter especially the hemicellulose and cellulose, still is a hinderance in its acceptability as human food. Much work has been done for the hydrolysis of cellulose, and a large number of cellulolytic micro organisms have been isolated for enzymatic hydrolysis of cellulose and hemicellulose [6]. High pressure steam treatment has been found to be quite effective for the partial removal of hemicellulose contents from different lignocellulosic materials [7]. Different strong alkalies have also been tried for the hydrolysis of cellulose and hemicellulose [8-10] but these are difficult to handle due to corrisive in nature. Therefore, attempts were made to use mild alkalies for the hydrolysis of polysaccharides particularly the cellulose and hemicellulose.

Present investigations were carried out to produce a detoxified seed meal low in cellulose and hemicellulose contents, so that it may become suitable as a source of dietary fibre for human consumption by applying various physicochemical and biological treatments.

Materials and Methods

The mustard seed meal (defatted mustard seed cake) was ground to 80 mesh size and was detoxified by enzymic treatment to eliminate allylisothiocyanate [4], while reduction of phytic acid was carried out by Niazi *et al.* method using 4% NaCl solution [3]. Detoxified meal was subjected to following treatments to reduce fibre contents:

High pressure steam treatment. Detoxified mustard seed meal having 20% moisture was autoclaved at different pressures (15 and 20 lbs/inch^2) for different intervals of time (15 and 30 mins). After the reaction time, pressure was gradually released to atmospheric pressure and then the substrate was dried at 100° for 4 hrs.

Alkali treatment. The materials was thoroughly mixed with the solution of sodium carbonate of sodium bicarbonate of different concentrations (0.5, 1.0, 1.5M), keeping 20% moisture contents and then it was placed in air tight glass jars at 50 ± 5° for 10 days. After which the glass jars were opened and exposed to air. Finally the treated material was dried at 100° for 4 hrs.

Alkali-steam treatment. The material was thoroughly mixed with the solution of sodium carbonate or sodium bicarbonate of different concentrations (0.5, 1.0, 1.5M) and then subjeted to high pressure steam treatment for a period of 30 mins as described above.

Biological treatment. Locally isolated culture of *Trichoderma viride* was propagated in Reese medium [11] containing 2% glucose and 2% agar. Three days old culture was transferred from the slants to inoculate 50 ml of sterlized Reese medium in a 250 ml erlenmeyer flask and incubated at 30° for 3 days on a rotary shaker with 120 r.p.m. and it was used as inoculum. Fifty grammes of meal was sterilized in a 500 ml erlenmeyer flask keeping water to substrate ratio 2:1 (v/w). 10 ml inoculum was added in each flask and fermentation was allowed to proceed at 30 ± 2° with shaking for different time periods (24, 48, 72, 96 hrs). After which, the material was dried at 100° for 4 hrs.

Chemical analysis. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose contents were determined as described by VanSoest and Wine [12] whereas cellulose was estimated by the method of Kurschner and Hanak [13] using nitric acid and acetic acid mixture. Reducing sugars in the extract of the sample were meassured by DNS method as described by Miller [14]. protein digestibility of the samples was estimated in vitro after digestion with pepsin-HCl solution [15].

Results and Discussion

High pressure steam treatment. It is apparent from the results reported in Table 1. that the neutral detergent fibre (NDF) especially hemicellulose was reduced by 18.16% when the meal was autoclaved at 15 lbs pressure for 15 mins. However, it was further reduced to 28.32% after autoclaving the meal at 15 lbs pressure for 30 mins. Increase in autoclaving pressure from 15 to 20 lbs did not result further reduction in NDF as well as hemicellulose even when the treatment lasted for 30 mins. Acid detergent fibre (ADF) and cellulose remained almost unchanged. The amount of reducing sugars was increased by 48.64% at 15 lbs pressure and 64.35% at 20 lbs pressure for 30 mins treatments. This indicates the conversion of hemicellulose into simple sugars as already had been reported by Ellis *et al.* [16]. An improvement in the digestibility of protein from 65.69 to 69.92% (by 6.43%) was observed after this treatment. Danke *et al.* [17] also observed an improvement in protein digestibility of cotton seed meal after autoclaving at 121° for 45 mins.

Effect of alkali treatment. Cellulose and hemicellulose contents of the meal were reduced by 23.13 and 26.73% respectively when it was treated with 1.5 M sodium carbonate. However, reduction in cellulose and hemicellulose contents was found to be 14.40 and 23.61% respectively on treatment with 1.5M sodium bicarbonate (Table 1). These treatments also decreased NDF and ADF of the meal. There was an increase in reducing sugars by 45.61% with 1.5M sodium carbonate and 33.53% with 1.5 M sodium bicarbonate treatment. Improvement in protein digestibility was 13.60 and 11.63% when meal was treated with 1.0 M sodium carbonate and 1.0 M sodium bicarbonate respectively. The digestibility

TABLE 1. EFFECT OF VARIOUS PHYSICO-CHEMICAL TREATMENTS ON THE REDUCTION OF FIBROUS COMPONENTS FROM DETOXIFIED MUSTARD SEED MEAL.

Alkali treatment	High pressure steam treatment		(%)*					
Concentration of alkali	Pressure[2] labs/inch	Time (mins.)	NDF	ADF	Hemi cellulose	Cellulose	Reducing sugars	Digestible protein
Untreated meal	–	–	59.63	30.71	28.92	22.00	3.31	65.69
–	15	15	54.36	30.67	23.67	21.73	4.09	67.00
–	15	30	51.42	30.59	20.73	21.85	4.92	69.92
–	20	15	50.82	30.55	20.11	21.55	5.21	68.90
–	20	30	50.57	30.58	19.86	21.49	5.44	69.88
0.5 M $NaHCO_3$	–	–	57.17	30.46	20.71	21.75	3.52	70.01
1.0 M $NaHCO_3$	–	–	49.21	27.06	22.15	18.35	4.40	73.33
1.5 M $NaHCO_3$	–	–	49.63	27.54	22.09	18.83	4.42	73.96
0.5 M Na_2CO_3	–	–	56.82	29.49	27.33	20.78	4.30	72.06
1.0 M Na_2CO_3	–	–	47.56	25.96	21.60	17.25	4.79	74.63
1.5 M Na_2CO_3	–	–	46.81	25.62	21.19	16.91	4.82	74.91
0.5 M $NaHCO_3$	15	30	40.26	23.15	17.11	14.44	6.11	78.63
1.0 M $NaHCO_3$	15	30	34.99	22.40	12.59	13.69	6.32	80.00
1.5 M $NaHCO_3$	15	30	33.48	20.93	12.55	12.22	6.57	80.73
0.5 M Na_2CO_3	15	30	36.39	21.34	15.08	12.63	6.66	79.99
1.0 M Na_2CO_3	15	30	33.54	22.22	11.32	13.15	6.73	81.73
1.5 M Na_2CO_3	15	30	32.52	21.06	11.40	12.35	6.80	82.22

* Average of three replicates.

of the detoxified mustard seed meal was found to be almost the same at higher levels of alkali.

Combined effect of alkali and steam treatment. This treatment was found to be the most effective for reducing the fibrous components of detoxified meal. NDF and ADF were reduced by 45.46 and 31.84% respectively on treatment with 1.5 M sodium carbonate and subsequent autoclaving at 15 lbs pressure for 30 mins. (Table 1), while cellulose and hemicellulose contents of the meal were reduced by 44.45 and 60.85% respectively alongwith twofold increase in reducing sugars and improvements (25.16%) in the digestibility of the meal protein. Similar results were obtained with sodium bicarbonate but to a lesser extent than sodium carbonate. The observations of Heller *et al.* [18] revealed that most of the cell wall material was solublized by refluxing wheat bran in the presence of sodium carbonate. Walter *et al.* [19] also prepared edible fibre from apple pomace after refluxing with 1.5% sodium hydroxide solution.

Biological treatment. Neutral detergent fibre particularly the cellulose contents were reduced to some extent by the propagation of *Trichoderma viride* on detoxified mustard seed meal (Table 2). These results indicate that there was linear relationship between reduction in cellulose contents and time of incubation with cellulolytic micro-organisms. Maximum reduction in cellulose contents was found to be 26.45% when the meal was fermented with *Irichoderma viride* for 72 hrs. However, there was no further reduction in cellulose content beyond 72 hrs fermentation. A slight decrease in neutral detergent fibre and acid detergent fibre was observed during this treatment. About 31% increase in reducing sugars was also observed which might be due to partial conversion of cellulose into simple sugars. There was no change at all in hemicellulose contents due to biological treatment of the meal. Protein digestibility was slightly improved after propagation of *Trichoderma viride* on the meal. Improvement in protein digestibility was only 4.88% after 72 hrs. No further improvement in protein digestibility was observed beyond 72 hrs fermentation of the meal. Improvement in protein digestibility may be attributed to the removal of polyphenolic compounds and unwanted polysaccharides from the mustard seed meal. Partial removal of polysaccharides probably created large spaces within the matrix, which allowed the proteolytic enzymes to attack easily on protein profile available in the detoxified mustard seed meal and consequently increased the digestibility of the protein.

TABLE 2. EFFECT OF BIOLOGICAL TREATMENT ON THE REDUCTION OF FIBROUS COMPONENTS FROM DETOXIFIED MUSTARD SEED MEAL.

Incubation time (hrs) with *T.viride*	NDF	ADF	Hemicellulose	Cellulose	Reducing sugars	Digestible protein
	(%)*					
Untreated meal	59.63	30.71	28.92	22.00	3.31	65.69
24	56.27	28.86	27.41	20.15	3.40	64.40
48	55.06	27.43	27.63	18.72	3.65	66.73
72	52.40	24.89	27.51	16.11	4.39	68.90
96	52.43	24.82	27.61	16.11	4.33	68.11

*Average of three replicates.

The meal obtained after treatment with sodium carbonate and autoclaving contained 33.5% NDF, 11.3% hemicellulose and 13.5 cellulose which are almost equivalent to food grade wheat bran values as reported by Anderson and Clydesdale [20], while the physical characteristics indicated that the product was taste-less, odour-less and brown in colour. Hence, the meal may be utilized as dietary source of fibre in different food preparations particularly in bakery products. However, further investiations are suggested to study the rhenological properties and subjective analyses of the food product containing treated meal before it is recomended for human consumption. It is further suggested that the meal may be treated with citric acid to neutralize the residual alkali prior to its addition to various food products.

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