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[Received October 9, 1984]

Protease Inhibitors and in vitro Protein Digestibility of Defatted Seed Cakes of Akashmoni and Karanja

B. MANDAL,* Chemistry Department, B. N. Mahavidyalaya, Itachuna P.O., Hooghly, West Bengal, India, and **S. GHOSH MAJUMDAR**, Chemistry Department, Burdwan University, and **C.R. MAITY**, Biochemistry Department, Burdwan Medical College, both at Burdwan 713 104, West Bengal, India

ABSTRACT

Trypsin and chymotrypsin inhibitor activities of defatted seed meals of akashmoni *(Acacia auriculaeformis)* and karanja *(Pongamia glabra)* were studied before and after detoxification. Protease inhibitor activities were significantly higher in the unprocessed seed meals when compared with the processed seed meals. Detoxification also improved the in vitro protein digestibilities of these seed meals significantly. Processed meals of these legume seeds, with lower protease inhibitor activities and higher protein digestibilities, could readily be used as animal feed.

INTRODUCTION

In order to solve shortages and high prices paid for traditional food grains, exploration of non-traditional seeds from different forest plants is imperative. Many unconventional waste seeds can be used profitably for this purpose, and the legume seeds have received much attention as they are good sources of protein and other nutrients. Although the seed proteins are of good quality and contain a number of essential amino acids, the main problem in the nutritional exploitation of these unconventional forest resources is the presence of antinutritional factors (1). Processing or detoxification of these seeds or their meals is necessary prior to their use in animal feed. The nutritive value and protein digestibility of these seeds generally are improved by processing to destroy the antinutritional factors (2). Among these factors, trypsin and chymotrypsin inhibitors have been studied in legume seeds (3,4). The polyphenolic compounds, generally referred to as tannins, also have received much attention (4,5). The presence of free toxic amino acids in legume seeds also should be considered in this context (6), as these antinutritional factors have been reported to reduce the biological value of protein by inhibiting the digestive enzymes; this greatly impairs the nutritive value of the seeds (4-7).

Akashmoni *(Acacia auriculaeformis)* and karanja *(Pongamia glabra)*, members of the family Leguminous, grow largely in India and have been recognized as potential minor non-edible oilseeds (8). India has the potential to produce over 111 thousand tons of karanja seed and 30 thousand tons of akashmoni seed annually, although hardly one-fourth of this capacity is used at present (9). Preliminary analyses (10), refining (11) and nutritional and toxicological evaluations (12) of these seed oils have been reported by this laboratory. Defatted seed meals of akashmoni and karanja have a high protein content, and their amino acid composition suggests they might be used as a valuable supplement in animal feeds (13-15). However, the feeding value of these seed meals is greatly impaired by the presence of antinutritional factors (14,16). In 1984, we devised a simple process for detoxification of karanja seed meal by refluxing the seed meal with 2% HCl. The resultant meal was fed to rats at 30% level of the diet for 30 days without identification of any toxic effects (17). Defatted seed meal of akashmoni also was detoxified by the treatment with lime and subsequent autoclaving; the processed meal, when fed to rats at a 30% level in the diet for 4 weeks, did not reveal any abnormality in growth or in blood or liver biochemical or histopathological characteristics (18). However, additional studies are needed to investigate the performance of these defatted seed meals prior to and after detoxification before they can be recommended as safe for livestock consumption. In this paper, the levels of protease inhibitors and results of in vitro protein digestibility of defatted seed meals of akashmoni and karanja before and after detoxification are reported.

MATERIALS AND METHODS

Materials

Seeds of akashmoni *(Acacia auriculaeformis)* and karanja *(Pongamia glabra)* were collected from the local forests of Burdwan, dried and defatted in a soxhlet distillation apparatus using hexane. The defatted meals were air dried to remove the traces of the solvent and stored at a low temperature until use. Trypsin (E.C. 3.4.21.4, 199 μ/mg) and chymotrypsin (E.C. 3.4.21.1, 61 μ/mg) (Worthington Biochemical Corporation, New Jersey), pepsin, pancreatin and casein (Sigma Chemical Co., St. Louis, Missouri) were used. Other chemicals and solvents used in this study were of AR grade.

Detoxification Process

For the detoxification of akashmoni seed meal, 500 g of the defatted seed meal was mixed thoroughly with 30 g of lime and subsequently autoclaved at 110 C for 8 min, then dried, cooled and stored until use. The methodology was

*To whom correspondence should be addressed.

detailed in our earlier publication (17). Karanja seed meal was detoxified according to our earlier method (18). In this process, 500 g of the seed meal was refluxed with 2 liters of 2% HCl for 5 hr. The meal was then cooled, and 45 g of NaOH dissolved in 100 ml of water was added to the slurry with mixing. The slurry was dried in a flat tray in a hot air oven and the dried meal was stored cold for experimentation.

Methods of Analysis

Total nitrogen content was determined by the micro-Kjeldahl procedure (19), and the crude protein content was calculated using a factor of 6.25.

Trypsin Inhibitor Activity

The trypsin inhibitor activity (TIA) was assayed following the method of Kakade et al. (20). Trypsin inhibitor was extracted by shaking 200 mg of the seed meal with 10 ml of 0.1 M phosphate buffer (pH 7.6) at room temperature for 1 hr. After diluting the extract four-fold, the aliquots of 0.2, 0.4, 0.6 and 0.8 ml were assayed for TIA. The protein content of the extract was estimated following the method of Lowry et al. (21), and the percentage of protein extracted was calculated.

Chymotrypsin Inhibitor Activity

Chymotrypsin inhibitor activity (CIA) was assayed following the method of Kakade et al. (22). For the extraction of chymotrypsin inhibitor from the seed meal, 0.1 M borate buffer (pH 7.6) was used. The extraction method was identical to that described above. Protein content of the extract also was determined (21).

In Vitro Protein Digestibility

A suitable amount of sample containing 6.75 ± 0.1 mg N was placed in a 50 ml conical flask, and 5 ml of dilute HCl (pH 2.0) containing 2 mg pepsin was then added to it. The container was incubated at 37 C for 16 hr in a water bath shaker. After the incubation period, 2 ml of pancreatin solution was added and the mixture was again incubated at 37 C for 24 hr. (For the preparation of pancreatin solution, 50 mg of pancreatin was dissolved in 100 ml of 0.1 M borate buffer [pH 6.8] containing 0.025 M $CaCl_2$ and the solution was filtered and used.) Two to three drops of toluene was added to the mixture during the incubation period, and the contents were stirred slowly on a mechanical shaker. After 24 hr of incubation, 7.0 ml of 10% (w/v) trichloroacetic acid (TCA) was added to stop the reaction, and the suspension was centrifuged at 10,000 × *g* for 15 min. The residue was washed twice with 5 ml of 5% TCA solution and the pooled supernatants made up to 25 ml with 5% TCA solution. A 5 ml aliquot was evaporated to dryness at 80-90 C, and the nitrogen content was determined by the micro-Kjeldahl procedure (19). The digestibility of each sample was calculated as the nitrogen in the sample supernatant minus nitrogen in enzyme blank supernatant, expressed as percentage of nitrogen in the starting material.

RESULTS AND DISCUSSION

Table I gives the protein content, trypsin and chymotrypsin inhibitor activities and protein in vitro digestibility values of defatted seed meals of akashmoni and karanja before and after detoxification. The results show that trypsin and chymotrypsin inhibitor activities were higher for the unprocessed seed meal of akashmoni, while the processed seed meal exhibited lower values for these protease inhibitors. The higher activities of unprocessed akashmoni seed meal might be due to the presence of polyphenolic compounds. It was observed that defatted seed meal of akashmoni contained 5.1% tannins, while seed meal that was lime-treated and subsequently autoclaved contained tannins at a level of only 0.4%. Tannins have been reported to be the antinutritional factors primarily responsible for the toxicity of defatted akashmoni seed meal (14,16). Lime treatment and autoclaving reduced the toxicity of seed meal by reducing the level of tannins. Simultaneously, the protease inhibitor activities also were significantly reduced. Trypsin and chymotrypsin inhibitor activities were positively correlated with the amounts of polyphenolic compounds, tannins. These results are in good agreement with the results of Milic et al. (23), who studied the activity of purified tannins on the trypsin digestion of casein. Different legume seed tannins also have been reported to inhibit the digestive enzymes (4-7,24). The low protein digestibility of unprocessed akashmoni seed meal might be due to its high level of polyphenolic compounds and protease inhibitors. Processing of this seed meal significantly improved the protein digestibility. In this context, this processing method did not adversely affect protein and nutrient contents and amino acid composition except for the 20-30% loss of lysine and sulphur-containing amino acids (17). Thus, processed seed meal of akashmoni with lower trypsin and chymotrypsin

TABLE I

Protein Contents, Trypsin and Chymotrypsin Inhibitors and Protein Digestibility of Defatted Seed Meals of Akashmoni and Karanja Before and After Detoxification

	Defatted akashmoni seed meal		Defatted karanja seed meal	
	Before detoxification	After detoxification	Before detoxification	After detoxification
Protein (%) N × 6.25	40.8 ± 2.6	40.1 ± 2.2	33.2 ± 1.84	32.0 ± 2.0
Trypsin inhibition				
Units/mg meal	20.2 ± 3.6**	2.5 ± 0.8	13.5 ± 1.1*	6.1 ± 0.9
Units/mg protein	84.6 ± 6.4**	8.8 ± 1.0	48.6 ± 2.5**	12.0 ± 1.2
Chymotrypsin inhibition				
Units/mg meal	11.0 ± 1.0**	2.1 ± 0.6	8.0 ± 1.9*	4.2 ± 0.6
Units/mg protein	34.6 ± 3.1**	6.5 ± 0.9	21.3 ± 0.8**	5.6 ± 0.8
In vitro protein digestibility (%)[a]	28.4 ± 3.2**	78.4 ± 5.2	34.2 ± 4.1**	72.5 ± 6.0

[a]Percent digestible nitrogen.

Values are mean of 6 determinations ± SEM.

Level of significance with respect to corresponding processed seed meal (Student's t-test): *P < 0.01; **P < 0.001.

inhibitor activities and tannins and higher protein digestibility would be readily used in animal feed.

Karanja seeds also are known to contain antinutritional factors, including karanjin, a furanoflavonoid (1.25%, $C_{18}H_{12}O_4$, m.p. 158.5 C); pongamol, a diketone (0.85%, $C_{18}H_{14}O_4$, m.p. 128 C); and glabrin, a complex amino acid (trace, $C_{21}H_{42}O_{12}N_3$, m.p. 290 C decomp) (25). Toxicity of these antinutritional factors and that of seed meal was extensively investigated (16,18). Destruction of these toxic compounds by acid hydrolysis processing was chemically verified (18). Earlier reports (16,18) of the merits and disadvantages of this processing method showed that nutrient composition and amino acid profile of karanja seed meal remained almost unaltered after processing. The present results show that this processing also reduced the levels of trypsin and chymotrypsin inhibitors and improved the protein digestibility significantly.

For the first time, this study reports the presence of trypsin and chymotrypsin inhibitors in the defatted seed meals of akashmoni and karanja and also demonstrates that suitable processing can reduce these protease inhibitor activities and improve the protein digestibilities of these seed meals.

ACKNOWLEDGMENTS

Sri B. Roy, Chemistry Department, Burdwan University, solvent extracted the akashmoni seeds. P. Banerjee, Chemistry Department, North Bengal University, provided technical assistance. This work was supported in part by research grants from the Indian National Science Academy, New Delhi (No. BS/RP-397/2620) to Sri B. Mandal.

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[Received December 27, 1984]

♣Effect of Triglycerides Containing 9,10-Dihydroxystearic Acid on the Solidification Properties of Sal *(Shorea robusta)* Fat

S. YELLA REDDY and **J.V. PRABHAKAR,*** Discipline of Lipid Technology, Central Food Technological Research Institute, Mysore 570 013, India

ABSTRACT

Components affecting solidification properties of sal *(Shorea robusta)* fat have been studied. Triglycerides containing 9,10-dihydroxystearic acid (DHS-TGs) present to about 3% have been found to affect the supercooling property of sal fat at as low a level as 2%. The DHS-TGs were composed of 57.5% stearic, 5.8% arachidic, 6% palmitic and 30.5% 9,10-dihydroxystearic acids. As DHS-TGs are soluble in acetone, solvent fractionation using acetone improved the supercooling capacity of stearin while that of the olein fraction was not affected. When the fat was subjected to dry fractionation at 35 C, DHS-TGs, due to their high melting nature, were removed to a greater extent in the form of stearin, thereby improving the supercooling capacity of the olein.

INTRODUCTION

Sal fat is one of the commercially important vegetable fats used in the manufacture of cocoa butter substitutes. It is similar to Borneo tallow in physical properties and is rich in 2-oleyl disaturated glycerides. In our studies on preparation of cocoa butter extenders, we noticed variations in the solidification properties of commercial sal fat samples (Fig. 1). This presented problems in maintaining uniform quality in sal fat fractions obtained from different batches of fats using one set of fractionation conditions. Preliminary studies indicated that some minor components present in refined and bleached sal fat affected its solidification properties. In this paper, the effect of one of the minor components, triglycerides containing 9,10-dihydroxystearic acid, on the solidification properties of sal fat is reported.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Refined and bleached sal fat received from M/s Specialty Fats Pvt. Ltd., Khamgaon, was used for this study. The fat (Type 2) had the cooling characteristics shown in Figure 1 curve a.