Effect of Irradiation on the Proteinase Inhibitor Activity and Digestibility (*in vitro*) of Safflower Oilcake

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Trypsin and chymotrypsin inhibitor activities of safflower oilcake were studied before and after irradiation. The various doses to which samples were exposed ranged from 7 Gy to 10 kGy. The trypsin inhibitor is inactivated at 42 Gy, whereas the chymotrypsin inhibitor remains active, even at the much higher dose of 10 kGy. The *in vitro* digestibility values also showed a significant improvement after irradiation. Exposure to a low dose of 42 Gy is sufficient to improve the nutritional value of the oilcake.

KEY WORDS: Antinutritional factors, detoxification, digestibility, *in vitro* studies, irradiation, nutritive value, oilseed residues, proteinase inhibitors, safflower cake, unconventional protein.

Oilcake, the residual matter left after the extraction of oil from oilseeds, has gained importance recently because of its high protein content, and is considered a potential source of unconventional dietary protein. The increasing global population is becoming a strain on conventional food supplies. Hence, more and more sources of protein not generally used are being explored for their nutritional properties. The use of unconventional sources of protein, such as oilcakes, is economically attractive because no additional land is required for production. The restricted utilization of these sources as human food or even as animal feed has been due to the presence of antinutritional factors (1). Prior to their use as food, it is thus necessary to detoxify the oilcake and improve both the organoleptic and nutritional quality (2). The toxic factors studied in a variety of oilcakes are proteinase inhibitors, polyphenols, aflatoxins, phytates and phytohemaglutinins (3). All these factors contribute to lowered palatability and biological value of protein which, in turn, reduces the nutritive value and thus the utilization of oilcake.

Safflower (*Carthamus tinctorius*) seed, primarily used for its oil, contains 35–40% oil, 17–22% protein and 35–40% hull (4). Plant breeders have successfully produced other varieties with varying hull-to-kernel proportion (5). A major factor for the growing demand of safflower oil in the form of cooking or salad oil is its high linoleic acid content (78%), which has some therapeutic value in preventing atherosclerosis (6,7). The oilcake protein increases to 40–60% from 17–22% protein in the seed (8), and is a good-quality protein with respect to essential amino acids (4). The quality of safflower kernel protein is comparable with soybean meal protein. Only lysine is critically limiting, and methionine and isoleucine are borderline. It contains enough histidine to meet the needs of children. Protein sources, such as milk, fish and legumes rich in lysine, will adequately supplement safflower protein. However, its use is limited due to the presence of toxic factors (9–11).

Solvent extraction has been the widely used method for detoxification, but we have tried to employ irradiation as a means to reduce, if not remove, the toxic factors. In the present paper, the effect of irradiation on proteinase inhibitor activity and the results of *in vitro* digestibility of the commercial safflower oilcake before and after irradiation are reported.

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MATERIALS AND METHODS

Commercial safflower oilcake was obtained from Shiva Shakti Oil Mills (Pune, India). The oilcake was irradiated for different doses of 7, 14, 28 and 42 Gy, as well as at 10 kGy with a ^{60}Co source at a dose rate of 0.17 Mrad/h.

Pepsin and trypsin were obtained from Sigma Chemical Co. (St. Louis, MO). Pancreatin was from Tuga Biochemical Labs (Pune, India). Other chemicals and solvents used in this study were of reagent grade.

Nitrogen analysis. Total nitrogen was determined by the Kjeldahl procedure (12) before and after irradiation.

Trypsin inhibitor activity. The trypsin inhibitor activity was assayed following the method of Kakade *et al.* (13). Trypsin inhibitor was extracted by grinding the sample in a mortar and pestle with phosphate buffer (0.1 M, pH 7.6) in the ratio of 1:20 at room temperature for 1 h. After centrifuging the suspension, supernatant aliquots of 0.2–1.0 mL were assayed for trypsin inhibitor activity. The protein content of the supernatant was estimated by Lowry *et al.'s* method (14).

Chymotrypsin inhibitor activity. Chymotrypsin inhibitor from the oilcake samples was extracted in 0.1 M borate buffer (pH 7.6). The extraction method was identical to that described above, and the inhibitor activity was determined by the method of Kakade *et al.* (15). Protein content in the extract was also determined.

Expression of activity. One unit of enzyme activity is defined as the amount of enzyme that converts 1 mg of protein to trichloroacetic acid (TCA)-soluble components in 15 min at 37°C and pH 7.6 (16). One unit of inhibitor activity is the amount of inhibitor that depresses the proteolytic activity by one unit.

In vitro *digestibility of proteins.* For trypsin digestion (17), 0.75 g of a samples was extracted for 30 min. in 0.1 N NaOH and neutralized, and then the volume was filled to 15 mL with water. To this, 3.75 mL of 1 M phosphate buffer was added to adjust the pH to 7.6. After preincubation for 15 min at 37°C, 0.75 mL of trypsin (1 mg/mL in 5 mM HCl, 1:250) was added. After trypsin addition, 4 mL of aliquot was removed, and 6 mL of 5% TCA was added to stop the reaction at 0, 2, 4 and 24 h of digestion. The solutions were left overnight for complete precipitation. Filtrates of each sample were used for estimation of TCA-soluble peptides by Lowry *et al.'s* method (14).

For pepsin followed by trypsin digestion (17), 0.75 g of an oilcake sample was extracted in 15 mL 0.1 N HCl for 30 min at room temperature. After preincubation for 15 min. at 37°C, 2 mL of pepsin (1 mg/mL in 0.1 N HCl) was added. After 2 h of digestion, the pH of the solution was adjusted to 7.6, and then 1 mL of trypsin (1 mg/mL in 5 mM HCl) was added. After 24 h of trypsin digestion, 4 mL of aliquot was removed, and 6 mL of TCA (5%) was added to stop the reaction. The solutions were left overnight for complete precipitation. Filtrates of samples were used for the estimation of TCA-soluble peptides.

For pepsin followed by pancreatin digestion (18), 0.75 g of an oilcake sample was extracted in 15 mL of 0.1 M HCl for 30 min at room temperature. After preincubation for 15 min at 37°C, 2 mL of pepsin (1 mg/mL in 0.1 M HCl; 1:3000) was added. After 4 h of pepsin digestion, the pH of the solution was adjusted to 7.6, then 1 mL pancreatin (1 mg/mL in borate buffer, pH 7.6, containing 0.025 M $CaCl_2$) was added, and the solution then kept at 37°C. Aliquots (3.5 mL) were removed after 10 and 22 h of digestion, and the reaction was stopped by the addition of 7 mL 10% TCA. The rest of the procedure was identical to that described above.

RESULTS AND DISCUSSION

The nutritional value of proteins depends on the presence of essential amino acids and their availability, *i.e.*, digestibility of the protein. The effective utilization of dietary proteins is thus the result of the action of proteolytic enzymes. The low nutritive value of plant proteins is due to the deficiency in one or more essential amino acids and to poor digestibility. Of the various antinutritional factors, it is largely the presence of proteinase inhibitors in the plant sources that inhibit the action of proteolytic enzymes (19). Trypsin inhibitor is most common in plants and has been extensively studied (20). Some work has also been done on chymotrypsin inhibitors (21,22).

To improve the nutritional value, complete or partial removal of these inhibitors is essential. Solvent extraction/heat treatment (17,23-25) has been the method of choice for removal of these inhibitors. Irradiation, which is being used only for preservation of food, is our method of choice for inactivating the proteinase inhibitors. Similar work has been reported recently for soybean seed (26).

The commercial oilcake was exposed to various radiation doses ranging from 7 Gy to 10 kGy, and their effect on the proteinase inhibitor activities was studied and confirmed by measurement of *in vitro* digestibility.

Table 1 gives the nitrogen content, trypsin and chymotrypsin inhibitor activities of the unirradiated and irradiated safflower oilcake. Total nitrogen content does not seem to be affected by irradiation. Similar observations also have been reported in soybeans (27). The proteinase inhibitor activities for both trypsin and chymotrypsin were higher for unirradiated oilcake than for irradiated samples. The decrease in protease inhibitor activity was directly proportional to the dose. A dose as low as 42 Gy was sufficient for complete loss of trypsin inhibitor activity. However, a dose of 10 kGy did not completely inactivate the chymotrypsin inhibitor. In soybeans, also the chymotrypsin inhibitor activity was less affected by irradiation than was the trypsin inhibitor activity (26).

Our sample (safflower cake) required only 42 Gy for complete inactivation of the trypsin inhibitor, a dose well below the advocated safe level (10 kGy), while soybean seeds showed a loss of only 25% in trypsin inhibitor activity at a dose as high as 100 kGy (27). This difference may be due to structural variation in the inhibitor itself or in a difference in the nature of the sample, *i.e.*, seed and cake.

The decline in proteinase inhibitor activity correlates well with the increase in the digestibility of the oilcake. The results for trypsin action, for pepsin followed by trypsin and for pepsin followed by pancreatin, are shown in Table 2. The change in digestibility of unirradiated and irradiated oilcake was observed in all three cases by the amount of TCA-soluble peptides released during digestion, indicating the presence of proteinase inhibitors. The digestibility of unirradiated samples ranged from 30 to 45%

TABLE 1

Inhibitor Activities of Unirradiated and Irradiated Safflower Oilcake[a]

Sample	Trypsin digestion		Chymotrypsin digestion	
	Units/mL protein	Units/mg protein	Units/mL protein	Units/mg protein
Unirradiated	2.94	0.968	2.68	0.344
7 Gy	2.33	0.768	n.d.[b]	n.d.
14 Gy	1.40	0.468	n.d.	n.d.
28 Gy	0.485	0.16	2.22	0.229
42 Gy	0	0	1.50	0.154
56 Gy	n.d.	n.d.	1.01	0.092
10 kGy	n.d.	n.d.	0.59	0.0525

[a]All values are the average of triplicate determinations.
[b]n.d., Not determined.

TABLE 2

Nitrogen Content and *in vitro* Protein Digestibility of Unirradiated and Irradiated Safflower Oilcake[a]

	Unirradiated	Dose of radiation				
		7 Gy	14 Gy	28 Gy	42 Gy	10 kGy
Total N (%)	6.08	n.d.[b]	n.d.	n.d.	6.02	6.08
In vitro protein digestibility (g/100 g protein)						
Trypsin digestion						
0 h	14.14	13.42	14.13	14.13	15.54	15.54
24 h	31.79	33.21	36.74	38.15	50.16	53.00
Pepsin followed by trypsin digestion						
0 h	11.41	n.d.	n.d.	n.d.	12.55	n.d.
26 h	45.58	n.d.	n.d.	n.d.	64.65	
Pepsin followed by pancreatin						
10 h	47.48	n.d.	n.d.	n.d.	56.65	n.d.
26 h	48.39	n.d.	n.d.	n.d.	63.92	64.43

[a]All values are the average of triplicate determinations.
[b]n.d., Not determined.

and of irradiated samples from 52 to 70%. The digestibility achieved by an exposure of 42 Gy is adequate, and a higher dose of 10 kGy increases the digestibility only marginally.

The results of *in vitro* digestion indicate the presence of proteinase inhibitors and confirm the loss in trypsin inhibitor activity by gamma irradiation at a low dose of 42 Gy. Even at 10 kGy, 100% digestibility is not achieved, indicating partial loss in the chymotrypsin inhibitor activity and presence of other antinutritional factors, such as polyphenols, tannins or phytates. Milie *et al.* (28) have reported the positive correlation between tannins and trypsin by studying the activity of purified tannins on the trypsin digestion of casein. Similarly, trypsin inhibitor activity and chymotrypsin inhibitor activity were positively correlated with the amount of tannins in akashmoni seed meals (18). Therefore, work has to be carried out to study the other factors responsible for the safflower oilcake digestibility limit of 55–65%.

The improvement in the digestibility values after inactivating the proteinase inhibitors in this study is quite comparable with solvent-extracted detoxified samples of akashmoni and karanja, wherein the digestibility obtained by pepsin followed by pancreatin digestion was 78 and 72%, respectively (18).

The processing technique with gamma irradiation did not affect the protein quality adversely. The overall results suggest that there is no harm in using irradiation for detoxification of oilcake because a low dose of 42 Gy is well below the permissible dose of 10 kGy. It has the advantage over other methods in that fewer steps are involved than for solvent extraction. Also, the chance of degrading the nutrients is less at this low-dose exposure than with use of strong solvents, such as HCl/NaOH or heat treatment.

This study reports the beneficial use of irradiation for detoxification of oilcake by inactivation of proteinase inhibitors, thus improving its nutritional value. Further work on other toxic factors is in progress.

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