Development of technology for detoxification of Indian mustard deoiled cake for poultry and livestock consumption

S. Sahni, S. Tickoo, Sindhu V. K., H.B. Singh

Mustard Research and Promotion Consortium 307, Jyoti Shikhar Building, District Centre, Janakpuri New Delhi-110058, India Email: sktickoo@rediffmail.com

Abstract

India produces almost 42 lakh tones of mustard annually with protein content of 37-38%. Mustard de-oiled cake (DOC) is the best and cheap source of protein for livestock and poultry in India, but is not being utilized properly as a direct feed for livestock due to the presence of antinutritional elements such as allylisothiocyanate, phenolic and phytic acid. The amount of allylisothiocyanate present in mustard DOC is 40-54 μmoles/gm and is sufficient to cause goiter in poultry. Phytic acid forms 2.3-2.5% of cake and phenolic make around 3.0-3.5% of it. Therefore, detoxification of mustard cake at this juncture is important for proper and efficient utilization of DOC as a livestock feed. But unfortunately, till date no such technology has been developed for its commercialization. In our research various experiments were undertaken to develop a technology for detoxification of mustard DOC. In first experiment, the DOC was treated with a solution of 10% NH_4OH and 1% ethanol at 80°C for half an hour followed by continuous washing with water. The quantification of various anti-nutrients was done following AOCS protocols. This detoxification process has shown promising results but cannot be used commercially as alcohol use will put huge pressure on industry's economic viability. In other experiment acetic acid treatments of DOC with simultaneous baking at 65°C was done for its successful detoxification where acetic acid (0.5% of DOC) treatment for 3 hours at 65°C with constant moisture level of 7% is sufficient to reduce the phytic acid up to as low as 1.91% and reduces the phenolic content up to 1.71%. In another experiment, the treatment of DOC with saturated steam at pressure of 15 psi found to be the most effective in its maximum detoxification, as the phytic acid concentration was reduced from 2.53% in original cake to 0.35% in cake after its treatment and phenolic concentration was reduced from 3.153% to 0.791%. The residual cake was found to be palatable for poultry, as it is free from the said antinutritional elements. The last experiment has the potential to be developed as technology for DOC detoxification and can be exploited for commercial use.

Key words: Detoxification, deoiled cake, allylisothiocyanate, phytic acid, phenolic, antinutritional elements, saturated steam.

Introduction

Mustard is used for production of high quality edible oil and a feed-grade meal. Mustard meal left after oil extraction from seeds is an excellent source of about 40% proteins, has reasonably well-balanced amino acid content (Ohlson, 1978) and favorable protein efficiency ratio (Delisle et al., 1984) is better than soy protein. Besides, so much of potential, utilization of mustard meal as a source of protein in livestock nutrition is limited due to the presence of allylisothiocyanate, phytic acid, and phenolic compounds. Presence of allylisothiocyanate in the diets leads to hyperthyroidism in animal and poultry, reduces the level of circulating thyroid hormones and alters the ratio between T3 and T4 in blood. Phenolic compounds contributed to the dark color, bitter taste and astringency of meals. They and/or their oxidized product also may form complex with essential amino acid enzymes and other proteins, thus lowering the nutritional value (Kumar et al., 1984). Phytic acid is a powerful chelator, especially of polyvalent cations, which are bound more strongly than monovalent cations (Graf, 1986) thereby markedly reducing the bioavailability of several multivalent cations by forming insoluble, phytate metal complexes. Moreover, the phytate also decrease protein digestibility through formation of indigestible protein-phytate complex. Therefore, much research has been directed towards detoxification of mustard meal from these antinutritional elements to make it palatable for livestock. The detoxification procedures investigated can be grouped into the following five categories: 1. Potential of the enzyme and removal of the hydrolytic products. 2. Inactivation of enzyme myrosinase. 3. Removal of antinutritional elements from mustard meal. 4. Destruction of antinutritional elements present in meal. 5. Breeding out antinutritional elements from crop. But till date not a single method has been developed which reduce all the antinutritional elements in a single treatment up to the extent which do not cause any detrimental effect to livestock. So, in this process we tried to develop a technology for detoxification of mustard deoiled cake without any further deterioration in the quality of meal.

Material and Methods

Phytic Acid Quantification: The phytic acid was extracted and determined according to the supernatant difference method of Thompson and Erdman (1982) with minor modifications. 1 gm sample was extracted with 25ml of 15% Trichloroacetic acid (TCA) containing 10% Sodium sulfate by mechanical shaking for 30 min followed by centrifugation at 12100x g for 15 min in REMI cooling Compufage CPR30. The supernatant was decanted and the pellet was re-extracted with another 25 ml for 30 min and again supernatant was decanted in previously obtained supernatant. The pellets were then collected together and phytic acid was precipitated with 1% $FeCl_3$ solution. The amount of phytic acid was obtained by the

difference of the phosphorus value between the initial supernatant and after precipitation with $FeCl_3$.

Allylisothiocyanate Quantification: The allylisothiocyanate were quantified according to Directorate General of Health Service Government of India protocol for allylisothiocyanate. In this process 5gm of mustard cake powder was taken in round bottom flask with 25 ml ethanol and 250 ml of glass distilled water and subjected to distillation using Liebig's condenser and the condensate was collected in a conical flask containing 25 ml 0.1 N $AgNO_3$ along with 10 ml 10% NH_4OH without any exposure to air till the solution in the flask made up to 150 ml. Along with it a blank was also placed. Then the solution was placed for aging using air column below 100^0C for 1 hr. After cooling make it to 200 ml by adding water. Out of this 100ml was taken and was titrated with 0.1N Ammonium thiocyanate till pink color develops. Volume of ammonium thiocyanate used was noted in sample as well as in blank.

Allylisothiocyanate content was quantified by using following formula:

$$\frac{9.915 \text{ X (Blank – Sample) X Normality of Solution used}}{\text{Weight of Sample}}$$

Phenolic Quantification: Phenolic in mustard meal was qualitatively tested using alcoholic $FeCl_3$ solution. About 2 g sample was extracted with ethanol (1: 6 w/v) for 1hr and the extracted volume was reduced in vacuum. The reduced volume was tested with alcoholic $FeCl_3$ solution. Presence of phenolic is indicated by a green color. The quantitative value of phenolic was obtained by finding the optical density at 230nm using the UV-VIS Spectrophotometer against the standard.

Detoxification Process: The detoxification of DOC has been done using three different methods as:

a. *Treatment with acetic acid*: In this process DOC was crushed and divided into 10 equal parts of 500 gm each. Mustard DOC was baked at 65°C and the moisture was maintained at 4% the product was then sprayed with acetic acid solution of concentrations 0.1-0.5%. The DOC powder was then kept overnight and on the next day it was subjected to mild steam and was again baked at same temperature for different time periods (1-3 hr) and final moisture level was maintained at 6.5%.

b. *Treatment with Ammonium hydroxide and ethanol*: In this process the material was immersed in a solution containing 10% ammonium hydroxide and 1% ethanol. The water and cake ratio was made to 1:10; the reaction mixture was kept at 80°C for half an hour. The material after separating from this solution was washed with fresh water. It took about 10 washings to properly remove maximum possible ethanol and NH_4OH mixture. The material was then centrifuged with industrial centrifuge to remove maximum possible water and was dried to 6% moisture level under hot air.

c. *Treatment with Saturated Steam:* This process was conducted by exposing the `DOC to the saturated steam at different pressures from 10-20 psi by employing autoclave unit after treating it with acetic acid. After treatment with steam DOC was washed with fresh water and then subjected to hot air oven to remove the water. Then DOC was dried for 2.5 - 3.0hr in oven as the moisture percentage came down to 4 -5% considering its storage view point.

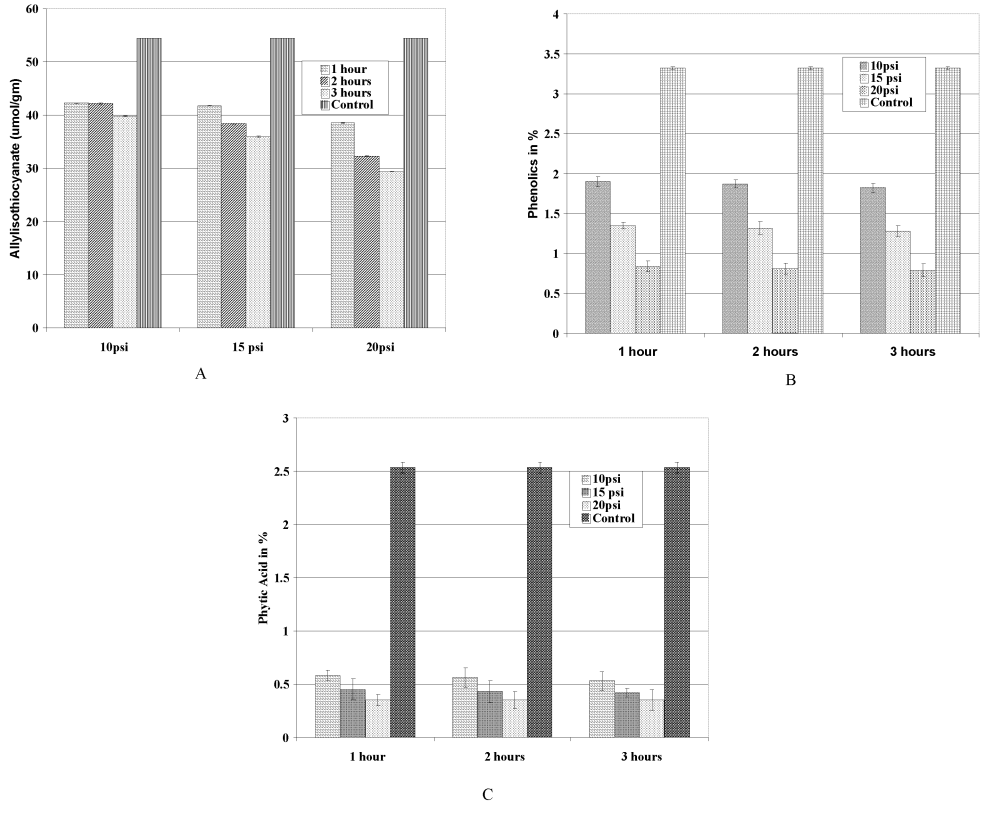

Fig.1. Amount of various antinutritional elements left in DOC after treatment with saturated steam and acetic acid treatment at different pressures. A. Amount of Allylisothiocyanate in µmol/gm, B. Phenolic % and C. Phytic acid % ; Fisher's test, $P < 0.05$, n (no. of samples) = 9.

Results

Acetic Acid Treatment: The phytic acid content was reduced to 1.91%, 1.90%, and 1.89% when baked for 1,2 and 3 hours respectively, the percentage of phenolic was found 1.75%, 1.74% and 1.71% respectively, allylisothiocyanate content in DOC also followed the same trend shown by phytic acid and phenolic. Its content after treatment for 3hr reduced to 28 µmoles from 52 µmoles per gram of DOC after drying.

Alcoholic ammonium hydroxide treatment: The amount of phytic acid in DOC was reduced to 2.11% of DOC, allylisothiocyanate reached the concentration of 41µmol/gm and DOC released about 50% of phenolic after the treatment with alcoholic ammonium hydroxide.

Saturated steam treatment: The amount of allylisothiocyanate left after treatment with acetic acid (0.5% of DOC) at 10, 15, 20 psi for 1, 2 and 3 hours in 9 different treatments were found to be 42.23, 42.11, 39.78, 41.72, 38.32, 35.85, 38.46, 32.19 and 29.33 µmoles per gm of DOC respectively. The amount of phenolic under the same treatment was found to be 1.90, 1.87, 1.82, 1.35, 1.32, 1.28, 0.84, 0.81 and 0.79% and phytic acid left in DOC after treatment was 0.58, 0.56, 0.53, 0.45, 0.43, 0.42, 0.35, 0.35, 0.35% with respect to the treatment.

Discussion

In present investigation three different treatments were tried on mustard DOC to make it palatable to livestock by reducing the content of various antinutritional elements. The first treatment of DOC was with acetic acid along with baking, which reduced the amount of various antinutritional elements up to great extent. This result is in accordance with Fallon (1995) who found the neutralization of phytic acid up to a great extent when treated with acetic acid. Colbin (1996) also observed the same results. Moist heat treatment drastically decreased the glucosinolate content (Schone et al., 1994). In second treatment process, the DOC was treated with ethanolic solution of Ammonium hydroxide that reduced the toxic materials abruptly. Our observations in this treatment got support with the findings of Shahidi et al. (1992) who determined the impact of this treatment on the reduction of allylisothiocyanate. Barlett (1998) also found reduction of toxicity in mustard meal after alkaline heating. In last experiment we treated mustard DOC with saturated steam at different pressures which resulted in comparatively a larger amount of removal of antinutritional elements without any visual loss in its texture or nutritional value ($P < 0.05$). This method was a slight modification of the method followed by Pawar and Palikar (1989) that soaked the pearl millet in water for reducing polyphenols and phytates.

Conclusion

In this study we tried to develop a commercially viable technology for detoxification of mustard oil cake by involving various methods either to neutralize or to remove the toxic substances so that it can be used as a high protein meal for livestock. We employed three treatments and all of them are efficient in removing the anti- nutritional elements but out of these we found the treatment of DOC with steam is the most suitable and commercially viable as it make the cake ready to eat feed for livestock and there is no need of huge investment in generating steam as the exhausted steam of the oil extraction unit can be used where as treatment with acetic acid make the food unpalatable and ammoniated alcohol treatment resulted in great loss of protein so is not commercially compliant. Moreover, the statistics of the results has shown that the treatment at 15 psi for 2hours is best as it does the detoxification maximally and is viable in terms of energy consumption.

Acknowledgement

Authors are grateful to Technology Mission on Oilseeds, Pulses and Maize (TMOP) and Council of Scientific and Industrial Research (CSIR) for providing uninterrupted financial support for completion of this study.

References

Barlett J. E., Klopfenstein C. F., Leipold H. W. (1998): Alkaline heating of canola and mustard meals reduces toxicity for chicks. Plant Foods for Human Nutrition **52**, 9-15.

Butler, E.J., Pearson A.W., Fenwick G.R. (1982): Problems which limit the use of mustard meal as a protein source in poultry diets. Journal of the Science of Food and Agriculture **33**, 866-875.

Colbin, A. (1996): Food and Healing, New York: Ballantine Books.

Dabrowski, K., Sosulski, F.W. (1984): Composition of free and hydrolysable phenolic acids in defatted flour of 10 oil seeds. Journal of Agriculture and Food Chemistry **32**, 128-130.

Delisle, J., J.Amiot, G. Goulet, C. Simard, G.J. Brisson, J.D.Jones. (1984). Nutritive value of Soybean, mustard and wheat flours in the rat Qual. Plant.-Plant Foods and Human Nutrition **34**, 243-251.

El Nockrashy, A.S., Mukherjee, D.K., Mangold, H.K. (1977): Mustard Protein Isolates by Countercurrent Extraction and Isoelectric Precipitation. Journal of Agricultural and Food Chemistry **25**, 193-194.

Fallon, S. (1995): Nourishing Traditions: The Cookbook That Challenges Politically Correct Nutrition and the Diet Dictorats: New Trends Pub Inc.

Graf, E. (1986). Chemistry and applications of phytic acid: An overview. Phytic Acid: Chemistry and Applications. E. Graf, ed. Pilatus Press, Minneapolis, MN, 173-194.

Krygier, K., Sosulski, F.W., Hogge, L. (1982): Free, esterified and insoluble phenolic acids. 2: Composition of phenolic in mustard flour and hulls. Journal of Agriculture and Food Chemistry **30**, 334.

Kumar R., Singh M. (1984): Tannins: Their adverse role in ruminant nutrition Journal of Agricultural and Food Chemistry **32**, 447.

Naczk, M., Shahidi,F. (1989) : The effect of methanol-ammonia-water treatment on the content of phenolic in canola. Food Chemistry **31**, 159-164.

Ohlson, R. (1978): Functional properties of Mustard oil and protein products: a survey. In: Proceedings of 5th International Mustard Congress, 152-167.

Pawar V.D., Parlikar G.S. (1990). Reducing the Polyphenols and Phytate and Improving the Protein Quality of Pearl Millet by Dehulling and Soaking. Journal of Food Science and Technology **27**, 140-143.

Shahidi F., Naczk M. (1992). An Overview of the Phenolic of Canola and Mustard. Chemical, Sensory and Nutritional Significance. Journal of the American Oil Chemists' Society **69**, 917-924.

Schone F., Kirchheeim U., Schumann W. (1994b). Glucosinolate degradation by mustard myrosinase and effect on mustard acceptability by growing pigs. Animal Feed Science and Technology **48**, 229-235.
Thompson D.B., Erdman J.W.Jr. (1982). Phytic Acid Determination in Soybean. Journal of Food Science **47**, 513-516.