A new method for preparation of non-toxic, functional protein hydrolysate from commercial mustard cake

Alireza Sadeghi Mahoonak[1], Bhagya Swamylingappa[2]

[1]Department of Food Science; Gorgan University of Agriculture, Science and Natural Resource, Iran
Email: sadeghiaz@yahoo.com
[2]Department of Protein Chemistry & Technology, CFTRI, Mysore, India – 570020

Abstract

Rapeseed/mustard is an important oilseed crop and ranks second in the world production of oilseed after soybean and second major oilseed crop in India. Traditionally, most of the mustard seeds are crushed for oil production and the cake obtained contains 34-40% protein, being used for animal feed or fertilizer because of the presence of glucosinolates, phytates, phenolics and fiber. In the present study, the defatted commercial mustard cake was fractionated to obtain low hull and high protein fraction. The fractionation reduced the fiber content by 60%. The low hull high protein fraction was used in the preparation of protein hydrolysate. The process comprises extraction of the protein in 0.1 M NaCl containing 0.1% ascorbic acid, incubation, extraction at optimum pH and treating with adsorbent.The protein was thermally coagulated, separated, washed and dissolved in water and treated with enzyme alkalase and spray dried. The yield of protein hydrolysate was 60% with a protein content of 80%. The protein hydrolysate had a very low content of isothiocyanate (0.12 mg/g) and oxazolidinethiones (0.09 mg/g) compared to the starting material (1.69 mg/g and 2.08 mg/g respectively). The reduction in isothiocyanate and oxazolidinethiones was 98.6 and 99.1%, respectively. The process resulted in the reduction of the phytate and phenolics by 98 and 97.5%, respectively and trypsin inhibitor activity totally inactivated. Nutritional evaluation indicated that the protein hydrolysate had better nutritional characteristics compared to starting material. Protein hydrolysate showed good functional properties with a solubility of more than 50% in all pH values.

Key worlds: Mustard cake, Protein hydrolysate

Introduction

Mustard/rapeseed is one of the major oilseed crops of India. Most of the seeds are used for oil extraction in Ghanies or expellers. In the traditional processing of mustard/rapeseed, the material is crushed without dehulling. The hull imparts dark color and contributes high amount of crude fiber (27%) in the meal. The oil obtained is dark in color and is not acceptable. The dark color cake finds very limited use in the food/feed purposes (Bell, 1984). The presence of toxic and antinutritional constituents such as glucosinolates, phytates, phenolics and hulls limits the use of rapeseed/mustard as a source of protein in food products. The glucosinolates are hydrolysed by the endogenous enzyme myrosinase to various toxic compounds that interfere with thyroid function and cause liver and kidney damage (VanEtten, Daxenbicher, 1977). Phytates are strong chelating agents that bind to polyvalent metal ions in the body including iron, calcium, magnesium and zinc rendering them unavailable for metabolism (Rutkowski 1971). Phenolic compounds impart bitter taste and dark color to the protein and its products. Tannins are the polyphenolics that complex with proteins suppressing the availability of essential amino acids (Sosulki, 1979). The use of rapeseed/mustard meal is limited in the diets of monogastric animals due to high content of indigestible fiber (Slominski, Campbell, & Guenter, 1994). Several detoxification methods including steaming, toasting, wet heating, water washing, microbial degradation and chemical treatment have been reported in the literature (Woyewoda, & Nakai & Watson; 1978; Maheswari, Stanley, & Gray, 1981). Membrane processing, dialysis, ultrafiltration, diafiltration, ion-exchange and protein micellar mass (PMM) methods for the preparation of protein isolates free of glucosinolates and low in phytates, phenolics and fiber have been reported (Tzeng et al., 1988b; Diosady, Tzeng, & Rubin, 1984; Tzeng, Diosady, & Rubin, 1988a; Tzeng, Diosady, & Rubin, 1990). The recovery of protein by aqueous extraction followed by isoelectric precipitation was low because of multiple isoelectric points. (El-Nockrashy, Mukherjee, & Mangold, 1977; Gururaj-Rao, Kantharaj-Urs, & Narasinga-Rao, 1978; Aruna & Appu-Rao, 1988). Recently, in our laboratory a process for the production of mustard protein isolate, with reduced toxic and antinutritional constituents for food and feed purposes, has been developed and patented (Alireza-Sadeghi, Appu-Rao, & Bhagya, 2004; Alireza-Sadeghi, Appu-Rao, & Bhagya, 2006). The use of heat adversely affects the functional properties of protein, therefore enzyme hydrolysis was used to improve the functional properties of protein.

Material and Methods

Protein hydrolystae was prepared according to the method developed and standardized in our laboratory (Alireza-Sadeghi, Appu-Rao, & Bhagya, 2006) with some modification to include enzyme hydrolysis step. Defatted commercial cake was fractionated to obtain three fraction with different hull contents, The low hull fraction was used for experiment. The low hull fraction of cake was dispersed in 0.1M NaCl containing 0.1% w/v ascorbic acid, in a ratio of 1:15 (w/v) and incubated at 37 ^{0}C for 30min. Then the pH was adjusted to 11 with addition of 1N NaOH. The dispersion was

subjected to shaking for 30 min at room temperature before centrifuging at 5000 rpm for 20 min. The pH of the supernatant was readjusted to 7.0 with 1N HCl. Activated carbon granules (2% w/v) were added and kept for shaking for 1h and filtered. Live steam was injected to the supernatant to raise the temperature to 93 ± 2°C for a period of 10 minatant was readjusted to 7.0 with 1N HCl. Activated carbon granules ()g for 30 min at room temperature before centr, cooled and centrifuged at 5000 rpm to separate protein. The coagulated protein after washing was dispersed in water to a solid content of 20% and adjusted to pH 8. Enzyme Alcalase (1ml/100g dry protein) was added and incubated at 50°C for 1h. After hydrolysis, the temperature was raised to 85°C for 10 min to inactivate enzyme, the solution was spray dried to obtain protein hydrolysate. The protein hydrolysate was analyzed for the presence of antinutritional factors and removal of them was calculated. The nutritional and functional properties of protein hydrolysates were calculated using appropriate methods.

Results

The chemical composition of whole seed, cake and its fractions are given in Table 1. The oil extraction by Ghani pressing increased the protein and crude fiber contents of cake from 25 to 32% and 11 to 14% respectively, compared to the starting seeds. The cake obtained after Ghani pressing still contained around 13% oil and was recovered by solvent extraction to obtain a defatted cake with low oil content. Removal of oil was beneficial during separation into three different fractions. The chemical composition of the fine and coarse fractions didn't show any significant differences (Table 1). These two fractions were combined and named as **"low hull high protein fraction"**.

Table 1. Chemical Composition of Mustard Seed, Cake and its Fractions

Constituents	Whole seed (Commercial Variety)	Cae	Low Hull Fine Fraction	Low Hull Coarse Fraction	High Hull Fraction
Moisture	6.3 ± 0.3	11.2 ± 0.2	8.8 ± 0.2	9 ± 0.1	9 ± 0.3
Protein (N × 6.25)	24.8 ± 0.4	32.3 ± 0.8	40.8 ± 0.2	40.6 ± 0.2	30.5 ± 0.5
Ash	3.7 ± 0.1	5.4 ± 0.2	6 ± 0.15	6.2 ± 0.1	5.5 ± 0.2
Fat	37.7 ± 0.1	13.2 ± 0.3	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
Crude Fiber	11 ± 0.5	14 ± 0.5	5.3 ± 0.2	5.5 ± 0.1	17 ± 0.5
Carbohydrate (by diff.)	16.5 ± 0.3	23.9 ± 0.5	36.9 ± 0.3	36.5 ± 0.2	35.8 ± 0.4

The high hull fraction contained low protein and higher amount of crude fiber (17%). Separation of hull increased the protein content and reduced the crude fiber content. The results showed that defatting and fractionation reduced the amount of crude fiber by 60 % and increased the amount of protein by 28 % compared to the starting cake.

The anti-nutritional factors presented in low hull fraction and protein hydrolysate are presented in Table 2. The content of isothiocyanate and oxazolidinethione in low hull fraction was 1.69 and 2.08 mg/g and it reduced to 0.12 and 0.09 mg/g in protein hydrolysate, respectively. The reduction achieved was around 99% as compared to low hull fraction of cake. The phenolics and phytic acid content of low hull fraction were 1.8 and 5.3%, respectively. In the protein hydrolysate, they were reduced to 0.15 and 0.35%, respectively. The reduction of these constituents was around 98% on the basis of protein yield. The trypsin inhibitor activity in low hull fraction was 3.8 TIU/mg of protein and completely inactivated in protein hydrolysates.

Table 2-Anti-nutritional Factors Present in Low Hull Fraction of

Constituents	Low Hull Fraction of Cake	Protein hydrolysate	Removal (%)
Isothiocyanates (mg/g)	1.69 ± 0.7	0.12 ± 0.01	98.6 ± 0.1
5-Vinyloxazolidine-2-thione (mg/g)	2.08 ± 0.04	0.09 ± 0.005	99.1 ± 0.1
Phenolics (%)	1.8 ± 0.05	0.15 ± 0.02	97.5 ± 0.2
Phytic Acid (%)	5.3 ± 0.08	0.35 ± 0.05	98 ± 0.2
Trypsin Inhibitor activity (TIU/mg protein)	3.5 ± 0.1	ND	100

Table 3.Nutritional Indices of Low Hull Fraction of Cake and Protein Hydrolysate

Parameters		Low Hull Fraction of Cake	Protein Hydrolysate from Cake
In vitro protein digestibility (%)*		80.6 ± 0.5	94.8 ± 0.2
C-PER		2.1	2.46
Essential Amino Acid Index		80.9	85.7
Predicted Biological Value		76.5	81.7
Nutritional Index		33.2	65.1
PDCASS	2-5 years old	68.8	69.2
	10-12 years old	90	91
	Adults	100	100
Available lysine (%)		4.45 ± 0.05	3.75 ± 0.05

Cake and Protein hydrolysate

The calculated nutritional indices of low hull fraction of cake and protein hydrolysate are presented in table 3. The increased i*n vitro* digestibility may be due to enzyme hydrolysis of proteins. All nutritional indices were higher in protein hydrolysate compared to low hull fraction of cake indicating higher nutritional value of protein hydrolysate.

Figure 1 show the protein solubility profile of cake protein hydrolysate (DH 8.7 ± 0.15) compared to protein concentrate and low hull fraction of cake at different pH values. The protein concentrate prepared from low hull fraction of cake (without using enzyme hydrolysis) had a solubility of 20-35% which increased to 50-65% on hydrolysis.

Table 2 shows the functional properties of low hull fraction of cake, protein concentrate and hydrolysate. In all cases, except foam stability, other functional properties improve due to protein hydrolysis compared to protein concentrate. Apparently, hydrolysate are capable of foaming but lack strength to maintain the foam as a result of reduction in molecular (peptide) size.

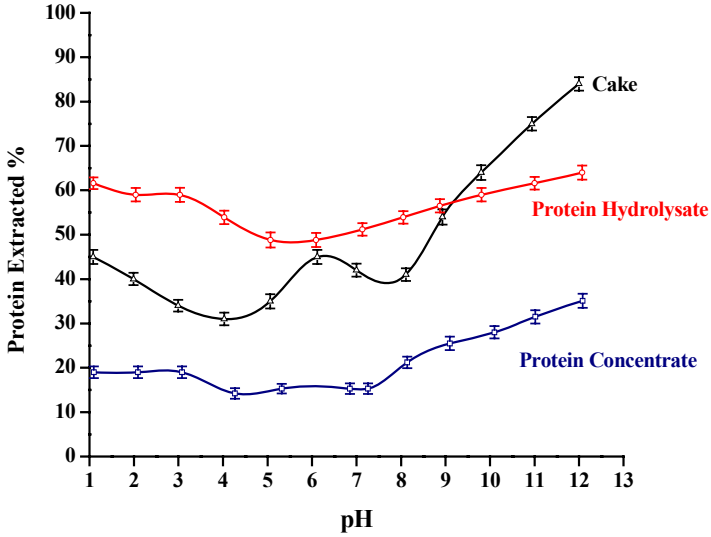

Fig 1. Protein Solubility Profile of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

Table 4. Functional properties of cake, protein concentrate and hydrolysate

Sample	Water absorption capacity g/100g	Fat absorption capacity ml/100g	Foam capacity %	Foam stability %	Emulsion capacity ml/g
Low hull fraction of cake	270 ± 5	160 ± 5	90 ± 3	75 ± 5	54 ± 1
Cake protein concentrate	204 ± 3	90 ± 5	55 ± 5	45 ± 3	31 ± 0.5
Cake protein hydrolysate	256 ± 5	132 ± 5	85 ± 5	40 ± 3	40.5 ± 0.5

Discussion

Different step of protein recovery including activated carbon treatment, heat coagulation and washing removes all antinutritional constituents with high yield and a protein hydrolysate with 80% protein content. Enzyme modification improves protein solubility and therefore positively affects other functional properties. The nutritional value expressed by different indices shows a high quality protein which can be used in human diet.

Conclusion

The detoxification process that used in this research removes more than 98 % of all antinutritional factors present in original material. The protein without hydrolysis showed good nutritional values but lack proper functional properties. Enzyme modification by alkalase improved functional properties and protein showed proper functionality for food uses. Therefore incorporation of enzyme modification in extraction method can improve protein functionality for food uses.

References

Bell, J.M. (1984). Nutrient and toxicants in rapeseed meal: a review. J. Anim. Sci., **58,** 996.

VanEtten, C.H., and Daxenbichler, M.E. (1977). Glucosinolates and derives products in cruciferous vegetables: total glucosinolates by retention on anion exchange resin and enzymatic hydrolysis to measure released glucose. J. Assoc. Off. Anal. Chem., **60**, 964.

Rutkowski, A. (1971). The feed value of rapeseed meal. J. Am. Oil Chem. Soc., **48**, 863.

Sosulski, F. (1979). Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: A review. J. Am. Oil Chem. Soc., **56**,711.

Slominski, B.A., Campbell, L.D., and Gunter, W. (1994). Oligosaccharides in canola meal and their effect on non-starch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci., **73**, 156.

Woyewoda, A.D., Nakai, S., and Watson, E.L. (1978). Detoxification of rapeseed protein products by activated carbon treatment. Can. Inst. Food Sci. Technol. J., **11**, 107.

Maheshwari, P.N., Stanley, D.W., and Gray, J.I. (1981). Detoxification of rapeseed products. J. Food Protec., **44**, 459.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1988a). Preparation of rapeseed protein isolate using ultrafiltration, precipitation and diafiltration. Can. Inst. Food Sci. Technol. J., **21**, 419.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1988b). Preparation of rapeseed protein isolate by sodium hexametaphosphate extraction, ultrafiltration, diafiltration and ion exchange. J. Food Sci., **53**, 1537.

Tzeng, Y.M., Diosady, L., and Rubin, L. (1990). Production of canola protein materials by alkaline extraction, precipitation and membrane processing. J. Food Sci., **55**, 1147.

Diosady, L.L., Tzeng, Y.M., and Rubin, L.J. (1984). Preparation of rapeseed protein concentrates and isolates using ultrafiltration. J. Food Sci., **49**, 768.

El-Nockrashy, A.S., Mukherjee, K.D., and Mangold, H.K. (1977). Rapeseed protein isolate by countercurrent extraction and isoelectric precipitation. J. Agric. Food Chem., **25**, 193.

Gururaj Rao, A., Kantharaj Urs, M., and Narasinga Rao, M.S. (1978). Studies on the proteins of mustard seed (B. Juncea). Can. Ins. Food Sci. Technol. J., **11**, 155.

Aruna, V., and Appu Rao, A.G. (1988). Isolation and characterization of low molecular weight protein from mustard (B. juncea). J. Agric. Food Chem., **36**, 1150.

Alireza Sadeghi, M., Appu Rao, A.G., and Bhagya, S. (2004). A process for the preparation of mustard protein isolate with reduced anti-nutritional factors. Indian Patent 480/DEL/04.

Alireza Sadeghi, M., Appu Rao, A.G., and Bhagya, S. (2006). Evaluation of mustard (Brassica juncea) protein isolate prepared by steam injection heating for reduction of anti-nutritional factors. Lebensm. Wiss. U. Technol., 39: 911-917.