STUDIES ON QUALITY IMPROVEMENT OF MUSTARD PROTEINS

A THESIS

Submitted to the

UNIVERSITY OF MYSORE

For the award of the Degree of

DOCTOR OF PHILOSOPHY
in
FOOD SCIENCE

By

Alireza Sadeghi Mahoonak
M.Sc. (Food Sci. & Technol.)

DEPARTMENT OF PROTEIN CHEMISTRY AND TECHNOLOGY
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE
MYSORE 570 020, INDIA

April 2006

T- 2090

ePrint@CFTRI

DEDICATED TO

MY WIFE BITA

& MY PARENTS

ePrint@CFTRI

DECLARATION

I hereby declare that the Thesis entitled "**Studies on Quality Improvement of Mustard Proteins**" submitted to the **UNIVERSITY OF MYSORE** for the award of **DOCTOR OF PHILOSOPHY IN FOOD SCIENCE**, is the result of the research work carried out by me under the guidance of **Dr. (Mrs.) BHAGYA SWAMYLINGAPPA**, Scientist, Department of Protein Chemistry and Technology, Central Food Technological Research Institute, Mysore, India during the period of 2002-2006. I further declare that the results presented in this thesis have not been submitted earlier for the award of any other degree or fellowship.

A.R.Sadeghi

Date: 12th April, 2006
Place: Mysore

(Alireza Sadeghi Mahoonak)

ePrint@CFTRI

Website : http://www.cftri.com Grams : FOODSEARCH, Mysore

☎ : 0821- 2514760, 2516802, 2514306 ; Fax : 0821 - 2517233

केन्द्रीय खाद्य प्रौद्योगिक अनुसंधान संस्थान, मैसूर - 570 020, भारत
Central Food Technological Research Institute
Mysore - 570 020, India

cftri

Dr. (Mrs.) Bhagya Swamylingappa
Scientist, Department of Protein Chemistry and Technology

April 12, 2006

CERTIFICATE

This is to certify that the Ph.D. thesis entitled ***"Studies on Quality Improvement of Mustard Proteins"*** submitted by ***Alireza Sadeghi Mahoonak*** for the degree of ***Doctor of Philosophy*** in ***Food Science*** to the ***University of Mysore***, Mysore, is the result of research work carried out by him in the ***Department of Protein Chemistry and Technology, Central Food Technological Research Institute, Mysore, India,*** under my guidance and supervision during the period 2002 – 2006. This work has not been submitted earlier, either partially or fully for the award of any degree or fellowship.

Bhagya Swamylingappa

BHAGYA SWAMYLINGAPPA
GUIDE

ePrint@CFTRI

Acknowledgment

I wish to express my sincere thanks and deep sense of gratitude to my guide Dr. (Mrs.) Bhagya Swamylingappa, Protein Chemistry & Technology , CFTRI, Mysore for her excellent guidance, invaluable suggestions and constant encouragement during the period of this Investigation. I am grateful to her for having believed in my abilities and providing me with the utmost independence at work during my research programme.

I wish to record my thanks to Dr. V. Prakash , Director, CFTRI, Mysore for his constant encouragement and valuable suggestions throughout the course of my work and providing me with an opportunity to utilize the facilities of the Institute and permitting me to submit the work in the form of a thesis.

I sincerely thank Dr. A. G. Appu Rao, Head, PCT, CFTRI, Mysore for his constant support, valuable suggestion and encouragement in the course of this investigation.

I also wish to thank Dr. M. C. Varadaraj, Head, HRD, CFTRI for his encouragement and support during my tenure at CFTRI.

I wish to express my sincere thanks to Dr. (Mrs.) Lalitha R. Gowda, Protein Chemistry & Technology, and Dr. (Ms.) Leelavathi. K. and Mr. M. Aalami, FMBCT, CFTRI for their help in amino acid analysis and product development.

ePrint@CFTRI

I thank to Dr. (Ms.) Uma V.Manjappara, Dr. Alok K. Srivastava, Mr. Jay Kant Yadav, and Mr. M. Obulesu, PCT, CFTRI for their help and cooperation during the preparation of the thesis.

I am extremely thankful to all staff of PCT, CFTRI, for making my stay here a wonderful and memorable experience.

I take great pleasure in putting my thoughts in words of gratitude to all staff of FED Pilot plant, CIFS, Photography Section and FOSTIS, CFTRI, Mysore for providing the facilities to carry out the research work.

I wish to express my gratitude to the Ministry of Science, Research and Technology, Iran and the Indian Council for Cultural Relations for the grant of scholarship and financial support during the course of my study.

Words are not adequate to express my gratitude to my parents, my wife Bita, and my daughter Sarvenaz for their support, patience, understanding and encouragement throughout the study.

A.R. Sadeghi

Alireza Sadeghi Mahoonak

ePrint@CFTRI

CONTENTS

ePrint@CFTRI

List of Tables

Cont…

ePrint@CFTRI

Cont…

ePrint@CFTRI

ePrint@CFTRI

List of Figures

Cont…

ePrint@CFTRI

Cont…

ePrint@CFTRI

Cont…

ePrint@CFTRI

ePrint@CFTRI

ABBREVIATIONS AND SYMBOLS

MT	Metric Tonn
mm	Millimeter (s)
cm	Centimeter (s)
µm	Micrometer (s)
°C	Degree Centigrade
sec	Second (s)
min	Minute (s)
h	Hour (s)
mm/g	millimole/gram
~	Approximately
M	Molarity
N	Normality
et al.	and Co-Workers
ml	Milliliter
w/w	weight/weight
w/v	weight/volume
v/v	volume/volume
g	Gram (s)
mg	Milligram (s)
kg	Kilogram (s)
rpm	Rotation Per Minute
Fig	Figure
AC	Allyl Cyanide
AITC	Allyl Isothiocyanates
PA	Phytic Acid
g/kg	gram/kilogram
mg/g	milligram/gram
mg/kg	milligram/kilogram
Da.	Dalton
CMC	Carboxy Methyl Cellulose
MPI	Meal Protein Isolate
DH	Degree of Hydrolysis

ePrint@CFTRI

TEMED	N,N,N′,N′-Tetramethyl-Ethylenediamine
BAPNA	Na-Benzoyl-DL-Arginine-ρ-Nitroanilide
FDNB	1-Fluro-2,4-Dinitrobenzen
A°	Angstrom
KDa.	Kilo Dalton
nm	Nanometer
µl	Micro liter
TIU	Trypsin Inhibitor Unit
TIU/mg	Trypsin Inhibitor Unit/milligram
TCA	Trichloroacetic Acid
L	Liter
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	SDS-Polyacrylamide Gel Electrophoresis
EAA	Essential Amino Acid
mtorr	Millitorr
kv	kiloVolt
WAC	Water Absorption Capacity
FAC	Fat Absorption Capacity
FC	Foam Capacity
FS	Foam Stability
EC	Emulsion Capacity
N/M^2	Newton/ Square Meter
gf	gram force
CS	Chemical Score
C-PER	Computed Protein Efficiency Ratio
NI	Nutritional Index
P-BV	Predicted- Biological Value
PDCAAS	Protein Digestibility Corrected Amino Acid Score
EAAI	Essential Amino Acid Index
ITC	Isothiocyanate
VOT	5-Vinyloxazolidine-2-Thione
SD	Standard Deviation
SEM	Scanning Electron Microscopy
µg	Microgram (s)
mA	MilliAmpere
nmol	Nanomole

ePrint@CFTRI

Mustard/rapeseed ranks second among the oilseed corps of the world after soybean with an annual production of 43 MT. India with an annual production of around 6.8 MT is one of the major producers of this oilseed (FAO, 2004). Mustard/rapeseed oil mainly used for edible purposes and the cake rich in protein is used for animal feed or as fertilizer. The presence of undesirable and toxic anti-nutritional constituents such as glucosinolates, phenolics, phytate and hull limit its use for food purposes. The high levels of these anti-nutritional constituents lead to nutritional disorder and toxicity in human beings as well as animals. The meal proteins apart from being rich in lysine contain adequate amounts of methionine which are limiting amino acids in cereal and oilseed proteins. The meal protein has a well balanced amino acid composition and the quality of the proteins is comparable to casein. Thus it may be considered as a potential source of food proteins. Considerable amount of work has been done on the isolation and detoxification of mustard/rapeseed proteins. However, none of these methods reduce or eliminate completely all the anti-nutritional factors in the protein. Therefore, these toxic and anti-nutritional compounds have to be removed as completely as possible before the protein could be used for human consumption. With this objective in view, a detailed investigation on these aspects has been carried out and this forms the subject matter of the thesis.

The thesis has been divided into four sections: (1) Introduction (2) Materials and Methods (3) Results and discussion, and (4) Summary and Conclusions.

In the “Introduction” section a survey of literature on the production of oilseeds including mustard/rapeseed in world in

ePrint@CFTRI

general and in India in particular, availability of mustard/rapeseed oil and cake, and the information available on the chemical composition of different variety of mustard grown in India are presented. Studies on the mustard protein and the anti-nutritional constituents present in the meal, and the various methods used to detoxify or eliminate these anti-nutritional factors have been described. Also, the scope and objectives of the present investigation are presented.

In “Materials and Methods” section, the source of materials and chemicals used and the experimental procedures employed in this investigation are described.

The “Results and Discussion” section has been divided into five chapters. Chapter 1 deals with the chemical composition and material balance of dehulled mustard meal prepared by dehulling, defatting and air classification of seed. This chapter also covers the chemical composition and yield data of low hull fraction of cake prepared by size reduction and separation of commercial cake. Chapter 2 deals with isolation and detoxification of protein from meal/low hull fraction of cake. This chapter includes the standardization of process for isolation and detoxification of protein. A detailed study on the role of each processing step in the preparation of protein isolate/concentrate free of toxic and anti-nutritional constituents has been conducted. The effect of processing on the chemical composition and removal of anti-nutritional constituents in isolate/ concentrate as compared to their starting material has been evaluated.

Chapter 3 describes the results of measurements on the physico-chemical and functional properties of proteins. These includes solubility of protein, water and fat absorption capacity, emulsion

ePrint@CFTRI

capacity, foam capacity and foam stability, color measurement, scanning electron microscopy and SDS-PAGE pattern of total proteins from meal, cake fractions, protein isolate, concentrate and hydrolysates. Chapter 4 deals with the nutritional evaluation of the proteins based on the amino acid composition data and *in vitro* digestibility of the proteins. The various methods used for the evaluation of nutritive value like essential amino acid index, predicted biological value, chemical score, nutritional index, computed protein efficiency ratio, protein digestibility corrected amino acid score and available lysine were described.

Chapter 5 deals with product development based on mustard protein isolate and its evaluation. A process was optimized for the preparation of spaghetti supplemented with different levels of mustard protein isolate. This chapter describes the effects of supplementation of spaghetti with mustard protein isolate and its effect on the chemical composition, cooking, sensory and color characteristics of spaghetti samples. The nutritional quality of enriched spaghetti was evaluated and compared to control spaghetti.

The “Summary and Conclusions” section describes the salient findings of this investigation and conclusions based on them.

The major findings of this investigation are:

Dehulling, defatting, size reduction and air classification reduced the crude fiber content from 10% to 3% and increased the protein content from 22 to 48% when compared to whole mustard seeds. Defatting and fractionation reduced the amount of crude fiber from 14% to 5.4% and increased the protein from 24.8% to 40.8% compared to cake.

ePrint@CFTRI

A new process has been developed for isolation and detoxification of protein from meal/cake involving alkaline extraction, activated carbon treatment, heat coagulation, washing and spray drying. Protein yield was 60%, the protein isolate had a protein content of 95% and negligible amounts of carbohydrate, fiber and ash. The protein isolate was light in color and bland to taste. Isothiocyanate content was reduced by 99.5% and the oxazolidinethione and trypsin inhibitor activity completely removed. Phytate and phenolics reduced by 99 and 98.7% respectively.

The isolation and detoxification of protein from low hull fraction of cake resulted in a protein concentrate with a protein content of 80% and yield was around 58%. The toxic hydrolyzed products of glucosinolates, isothiocyanate and oxazolidinethione were reduced by 98.6 and 99.1% in the protein concentrate, respectively. The phytate and phenolics were removed by 97.5 and 98%, respectively. The trypsin inhibitor activity was inactivated completely.

The modification of protein isolate/concentrate by enzyme alcalase improved the functional properties of protein. The use of ascorbic acid and removal of phenolics improved the color characteristics of different protein products. Scanning electron microscopy of different fractions showed protein bodies with different size which reduced on enzyme hydrolysis.

The SDS-PAGE pattern of meal, low hull fraction of cake, protein isolate and concentrate showed three major bands corresponding to molecular weight of 34,000; 28,000; and 20,000 Da. However the protein hydrolysates did not show bands corresponding

ePrint@CFTRI

to the above subunits as the proteins were hydrolyzed to small peptides.

The amino acid profile of protein isolate showed higher amounts of leucine, methionine and cysteine and lower levels of lysine compared to the meal. However, the amino acid composition of the protein hydrolysate prepared from meal did not show any difference to the protein isolate prepared from the meal. The *in vitro* digestibility and the calculated nutritional indices such as Computed Protein Efficiency Ratio (C-PER), Essential Amino Acid Index (EAAI), Predicted Biological Value (P-BV), Nutritional Index (NI), and PDCAAS of protein isolate and hydrolysate were higher than the dehulled meal. Protein isolate showed higher C-PER, EAAI, Predicted Biological Value and Nutritional Index compared to casein and the PDCAAS value for age group of 10-12 years old and adults were comparable to casein.

The low hull fraction of cake contained higher amounts of lysine and tryptophan, while the protein concentrate and hydrolysate contained higher amounts of leucine, isoleucine, cysteine, phenylalanine and tyrosine. The protein concentrate and hydrolysate from cake had greater *in vitro* digestibility, Chemical Score, Computed Protein Efficiency Ratio, Essential Amino Acid Index, Predicted Biological Value, Nutritional Index and PDCAAS compared to low hull fraction of cake.

The spaghetti prepared by supplementation of semolina with 2.5, 5 and 10% mustard protein isolate showed higher protein content. They also showed higher firmness and lower stickiness values compared to control spaghetti. The cooking loss, protein loss and

ePrint@CFTRI

cooked weight were decreased with increased supplementation levels. The results of the sensory evaluation of these spaghetti indicated that the color and texture scores were higher, while the taste and mouthfeel were lower with increased supplementation levels. The overall quality of spaghetti prepared with mustard protein isolate scored higher than control spaghetti. The essential amino acid composition of enriched spaghetti samples generally increased compared to control spaghetti. The nutritional indices of all the enriched spaghetti prepared with mustard protein isolate were higher than the control spaghetti.

ePrint@CFTRI

Mustard and rapeseed rank second among the seed crops of the world after soybean (Table 1) with an annual production of around 43 MT. India is one of the major producer of this oilseed (Table 2) and the annual production is around 6.8 MT (FAOSTAT, 2004). The principal producing countries are China, Canada, India, Germany, France, UK, Australia, Poland and USA (Table 3). Mustard and rapeseed are grown extensively in the north-eastern part of India in UP, Haryana, Punjab, Rajasthan, West Bengal and Bihar.

The principal varieties of rapeseed and mustard grown in India are *Brassica juncea,* commonly known as Rai or Laha, *Brassica campestris*, Brown Sarson and *Brassica campestris*, varieties toria. *Brassica juncea* is well adapted condiments of northern India and China, where brown and yellow seeded varieties are available (Rai et al., 2002).

Indian mustard (*Brassica juncea)* contains about 30-47% oil, 20-25% protein and 18-20% husk (Kantharaj Urs and Parpia, 1969). The oil from *Brassica* species are characterized by the presence of very high amount of erucic acid (C-22 monounsaturated fatty acid) constituting nearly 45-50 % of the total fatty acids. Varieties of rapeseed have been developed with lower erucic acid (<2%) content (Jones, 1976; Gunston, 2004). Low erucic acid varieties of *Brassica juncea* now exist but bulk of this crop is still high in erucic acid and oil from different rapeseed types continues to be accepted for human consumption in Asia. Mustard and rapeseed are grown principally for edible oil and industrial applications. The residue or cake left after oil extraction is used as animal feed.

ePrint@CFTRI

India was a minor exporter of rapeseed/mustard oil during 2000-2003 (Table 4 and 5). Although India is a major producer of this oilseed, but share of India in export of oil and cake is very low. This is mainly because of two reasons. First, mustard/rapeseed varieties commonly grown in India contain 40-50% erucic acid in the oil which is far in excess of the desired upper limit of less than 2%. Secondly is the presence of higher amounts of glucosinolates (80-160 mm/g) than the desired international level (<30 mm/g) in the oil free meal or cake which restrict the widespread use of Indian mustard/ rapeseed cake as animal feed.

ePrint@CFTRI

Table 1. Production of Major Oilseed in World and India during 2000-2004 (MT)

Year		Soybean	Rapeseed	Groundnut in shell	Cottonseed	Sunflower
2004	World	206,409,525	43,654,163	36,057,281	41,233,000	26,208,114
	India	7,000,000	6,800,000	7,500,000	4,800,000	1,250,000
2003	World	189,213,383	36,597,829	35,327,944	34,459,562	27,756,433
	India	6,800,000	3,918,000	7,700,000	4,689,000	1,086,000
2002	World	180,909,511	34,045,937	33,265,300	32,523,095	24,480,001
	India	4,558,100	5,082,600	4,362,800	2,964,000	901,900
2001	World	176,761,491	35,923,812	36,076,982	36,985,410	20,311,101
	India	5,962,706	4,187,200	7,027,500	3,400,000	679,500
2000	World	161,405,464	39,510,817	34,968,600	33,037,892	26,386,977
	India	5,275,806	5,788,400	6,480,300	3,281,800	646,400

Source: FAOSTAT, FAO, WHO

ePrint@CFTRI

Table 2. Rapeseed/Mustard Production in World and India (MT) and Share of India in World Production during 1995-2004

Year	World	India	Share %
2004	43,654,163	6,800,000	15.6
2003	36,597,829	3,918,000	10.7
2002	34,045,937	5,082,600	14.9
2001	35,923,812	4,187,200	11.7
2000	39,510,817	5,788,400	14.7
1999	43,182,675	5,663,900	13.1
1998	35,752,416	4,702,900	13.2
1997	35,068,083	6,657,900	19
1996	30,428,211	5,999,500	19.7
1995	34,185,196	5,758,000	16.9

Source: FAOSTAT, FAO, WHO

ePrint@CFTRI

Table 3.Major Rapeseed/Mustard Producing Countries During 2000-2004 (MT)

Country	2004	2003	2002	2001	2000
China	11,900,10	11,410,006	10,552,254	11,331,466	11,380,579
Canada	7,001,100	6,771,000	4,178,100	5,017,100	7,205,300
India	6,800,000	3,918,000	5,082,600	4,187,200	5,788,400
Germany	5,250,000	3,638,000	3,848,696	4,160,099	3,585,661
France	3,961,000	3,361,199	3,320,213	2,877,672	3,476,819
UK	1,612,000	1,771,000	1,468,000	1,157,000	1,157,000
Australia	1,549,000	1,622,000	871,000	1,756,000	1,775,000
Poland	1,292,329	792,971	952,737	1,063,638	958,145
USA	572,350	686,476	706,260	908,350	909,030

Source: FAOSTAT, FAO, WHO

ePrint@CFTRI

Table 4.Export of Rapeseed Oil During 2000-2003 (MT)

Country	2003	2002	2001	2000
Germany	553,385	939,983	888,141	770,976
Canada	499,891	458,178	550,202	570,190
France	229,385	278,289	161,299	239,783
Netherlands	210,858	259,295	228,968	222,840
Belgium	164,369	191,113	228,915	279,323
UK	269,321	161,859	47,243	62,305
USA	79,551	77,912	109,999	121,002
India	1,881	887	3,940	121,002

Source: FAOSTAT, FAO, WHO

ePrint@CFTRI

Table 5.Export of Rapeseed Cake During 2000-2003 (MT)

Country	2003	2002	2001	2000
Germany	1,117,559	1,151,430	1,225,638	1,092,633
Canada	1,126,716	764,951	998,171	1,194,941
Belgium	248,642	219,710	313,163	311,077
India	233,882	248,047	253,276	69,312
Poland	157,419	202,113	226,450	160,159
China	182,450	259,867	475,663	978,357
UK	191,726	284,881	116,151	195,076
France	77,057	79,942	47,484	41,896
USA	22,723	19,278	11,252	9,233

Source: FAOSTAT, FAO, WHO

ePrint@CFTRI

Composition of Rapeseed/ Mustard

Mustard/Rapeseed Oil

Commercially the mustard/rapeseed is processed in the oil mill and it yield 40% oil and a cake which contains about 50% protein. The major emphasis is on the oil which is priced several times higher than the by-product cake. The quality of the oils is chiefly characterized by their fatty acid composition and determines the value and utility of the oil (Maheshwari et al., 1981).

Presently the fatty acid profile of Indian mustard/rapeseed is inferior in quality compared to other vegetable oils. It contains very high amount of undesirable long chain fatty acids like eicosanoic and erucic acid. Indian mustard (*Brassica juncea*) contains palmitic acid 3%, oleic acid 12%, linolenic acid 13%, eicosanoic acid 7%, erucic acid 48% and others 4%. The *B. campestris* has also similar fatty acid profile (Ahuja, 1994). The European varieties of *B. napus* and *B. campestris* contain low erucic acid compared to the Indian varieties of *B. campestris* and *B. juncea*. Consumption of high erucic acid mustard/rapeseed oil in higher amounts could cause damage to heart muscle. High erucic acid in edible oil limits its use as food, but is desirable in the manufacture of lubricants, plastic, perfume etc.

Rapeseed/Mustard Meal

Rapeseed meal after oil extraction is an excellent source of protein and contains well above 40% protein (Table 6). Rapeseed proteins have very well-balanced essential amino acid composition which compared favorably with soy proteins and the FAO pattern of essential amino acid requirements for human adults.

ePrint@CFTRI

Table 6. Protein and Oil Content of *Brassica* Oilseeds

Brassica oilseeds	*Percent (dry basis)*		
	Oil	*Protein(N×6.25)*	
		Whole Seed	*Defatted Meal*
B.napus	44.7	23.8	*43.1*
B.campestris	42.7	23.8	*41.5*
B.juncea	*37.2*	*27.5*	*43.8*

Source: Maheshwari et al. (1981)

ePrint@CFTRI

The proteins are especially rich in lysine and methionine, which are limiting essential amino acids in most cereal and other vegetable proteins (El-Nockrashy et al., 1975a; El-Nockrashy et al.,1975b). The potential value of rapeseed proteins as one of the best oilseed proteins for supplementation of human diet is well documented (Lo and Hill, 1971a; Lo and Hill, 1971b; Girault, 1973; Sosulski and Sarwar, 1973; El-Nockrashy et al., 1975a; El-Nockrashy et al., 1975b). In addition, rapeseed protein products with desirable functional properties are acceptable for use in various food formulations for human consumption (Ohlson and Tear, 1974). The results of a collaborative study on protein quality by six laboratories indicated that the quality of rapeseed protein was similar to casein and superior to proteins from other vegetable sources such as soybean, pea and wheat (Sarwar et al., 1984). The mustard proteins have a protein efficiency ratio (PER) of 2.5-3.0 compared to soy, groundnut, cottonseed and sesame protein which are in the range of 1.5-2.5 (PAG Compendium, 1975; Venkat Rao et al., 1976).

The rapid increase in population and prevailing malnutrition in the highly populated developing countries have created need to find and use other sources of proteins. The adequate supply of protein depends on the increased utilization of proteins from oilseed and cereals. Now, there has been considerable interest in using rapeseed meal as a source of protein because of its high quantity and quality of protein, low cost and substantial availability.

The factors which favor the use of rapeseed protein in human nutrition are: 1. Rapeseed is cultivated in countries which do not have suitable agricultural or environmental condition for growing other oilseed (Ohlson, 1972); 2.The concentration of essential amino

ePrint@CFTRI

acids in rapeseed protein compares favorably with the amino acid requirements of human adults (FAO, 1975; El-Nockrashy et al., 1975a); 3.Rapeseed protein is characterized by relatively high content of lysine (Leslie and Summers, 1975; El-Nockrashy et al., 1975b) which is the first limiting amino acid in cereal and other vegetable protein and; 4.Rapeseed protein products with high nutritional value and satisfactory functional properties are well accepted (Ohlson and Tear, 1974).

Mustard/ Rapeseed Proteins

The proteins from *Brassica* species mainly consist of two protein fractions: a high molecular weight protein (12S fraction) and a low molecular weight fraction (1.3-1.8S) constituting about 25 and 70% of total proteins, respectively (Gururaj Rao et al., 1978; Gururaj Rao and Narasinga Rao, 1981; Mieth et al., 1983a; Prakash and Narasinga Rao, 1986; Aruna and Appu Rao, 1988).

Native albumins (1.3-1.8S fraction) are soluble in aqueous extraction mediums with low ionic strength, independent of pH value. The protein can be precipitated by salting out using ammonium sulphate, ethanol treatment,heat coagulation at isoelectric pH range (9.5-12), complex formation with polyanionic compounds (alginate, pectinate, carboxylate), flocculating agents (polyacids, polyamides and polyalcohol), tanning agents (tannins) and strong acids such as trichloroacetic acid, sulfosalicylic acid (Bhatty, 1972, Bhatty and Finlayson, 1973; Schwenke et al., 1973; Gillberg and Tornell, 1976a; Lonnerdal et al., 1977).

Native globulins (12S fraction) from *Brassica* seed are soluble at pH values above and below the isoelectric point in solvents of low

ePrint@CFTRI

ionic strength. The globulins are normally precipitated by pH adjustment to the isoelectric point, and in some cases by reduction of ionic strength (dilution or dialysis), salting out, and complexing or heat coagulation (Nagano and Okamoto, 1976; Lonnerdal et al., 1977; Simard et al., 1977).

Globulins

The 12S globulin is one of the main storage proteins in the seed of *Brassica* species (Bhatty et al., 1968; Goding et al., 1970; Finlayson et al., 1969; Finlayson et al., 1973; MacKenzie and Blakely, 1972; Prakash and Narasinga Rao, 1986). The 12S globulin was first isolated from *B. napus* by Bhatty et al. (1968). The defatted meal was extracted with 10% NaCl, dialysed and purified by gel filtration on Sephadex G-100. The 12S globulin of *B. Campestris* has been characterized and it was found to contain 12.9% (w/w) carbohydrate consisting of arabinose, galactose, glucose, inositol, glucosamine, and mannose. The observation that the rapeseed aleurone grains contain globoid bodies suggests the presence of phytic acid and could be related to the presence of inositol in the 12S component. The amino acid composition of the globulin indicates that it is high in acidic amino acids; glutamic and aspartic acids (Prakash and Narasinga Rao, 1986). The 12S mustard proteins dissociate into 3S fraction upon decreasing the pH from neutral to pH 3.6 (Bhatty et al., 1968). A part of the 3S component re-aggregated to 7S component with further decrease in pH to 2.2. Further, PAGE pattern of 12S protein showed at least four components, indicating that the molecule was an aggregate of subunits. Kishore Kumar Murthy and Narasinga Rao (1984) studied the effect of low pH on the association-dissociation and denaturation behavior of 12S globulin. The results of ultracentrifugation and

ePrint@CFTRI

electrophoresis experiments indicated that the protein dissociated into lower molecular weight fraction as the pH decreased from 5.0 to 3.0. The changes in the optical properties suggest the unfolding of the protein molecule. It was concluded that both dissociation and denaturation of protein occurred in the pH range 5.0 to 3.0 and below this pH, refolding and/or reaggregation were indicated.

Schwenke et al. (1983) have reviewed the structure of 12S globulin from rapeseed. From their studies, it was concluded that the 12S globulin of rapeseed is an oligomeric protein with a molecular weight of 300,000 and is composed of six subunits arranged in trigonal antiprism with point group symmetry of 32 (D_3). The protein consists of four polypeptide chains with different molecular weight in the range of 18,500 ± 800; 21,000 ± 400; 26,800 ± 900 and 31,200 ± 1,600. The behavior of the 12S globulin depends on various conditions such as ionic strength, pH, denaturants, etc. The 12S protein appears to be very sensitive to change in ionic strength and pH and dissociated to 7S. At high pH or in denaturant solution the 7S protein dissociated to a 3S or 2S component. The protein appears to be rich in glutamic and aspartic acids and is an acidic protein. There is a contradiction regarding the isoelectric point and the reported value ranges from pH 5.0 to 7.25. This may be due to the fact that nearly 11 species with isoelectric points ranging from 4.75 to 9.15 are detected in the protein. This could be also due to varietal differences and differences in the homogeneity of protein (Prakash and Narasinga Rao, 1986).

Albumins

The low molecular weight protein was first isolated by Bhatty et al. (1968) from *B. napus*. This low molecular protein fraction

ePrint@CFTRI

sediment as a single peak in analytical ultracentrifugation with a sedimentation coefficient of 1.3-1.8 S. Lonnerdal and Janson (1973) have shown that the proteins have four fractions and are basic in nature with molecular weight ranging between 12,000 to 14,000. The proteins have similar amino acid composition and each protein consist of two chains, held together by two disulfide bridges. Glycine is the terminal amino acid in all four proteins.

Schwenke et al. (1973) isolated and characterized the rapeseed (*B. napus*) albumins. The molecular weight determined by various methods was 14,400 ± 400. The amino acid composition revealed that the protein contain relatively high amount of cysteine (6.9%) and lysine (9%). The secondary structure of the protein consisted of α-helix, 40 to 46%; β-structure, 11 to 16%; and aperiodic structure, 41 to 43%. Compared to the 12S protein fraction, the 2S component appears to be a basic protein. It has high content of cysteine and lysine with an isoelectric point of 10.0. The high content of α-helix, nearly 45%, compared to 5% in 12S fraction is another characteristic of this fraction (Prakash and Narasinga Rao, 1986). The high degree of amidation of the glutamic and aspartic acids is responsible for the strongly basic character of low molecular weight rapeseed proteins (Lonnerdal and Janson, 1973).

Albumin fractions contain functional proteins such as enzymes or enzyme inhibitors, which are distinguished by the presence of strongly ordered structure. Trypsin-inhibiting activity could be detected these fractions (Gururaj Rao and Narasinga Rao, 1981; Mieth et al., 1983a).

ePrint@CFTRI

Mustard/rapeseed protein is of excellent nutritional quality. However its use is limited due to the presence of anti-nutritional and toxic factors such as glucosinolates and their hydrolysis products, phenolics, phytates and hull. The effect of these anti-nutritional factors on the nutritional and physico-chemical properties of protein is presented in the following pages.

Glucosinolates

The glucosinolates are a large family of sulfur containing compounds found in family of c*ruciferae* such as crambae, kale, mustard, rape, cabbage, turnip etc (Ahmed, 1972; VanEtten et al., 1969). All the glucosinolates possess a common basic structure comprised of a β-D-thioglucose group, a sulfonated oxime moiety and a variable side chain derived from methionine, tryptophan, phenylalanine and some branched chain amino acids. The general structural formulae of the glucosinolates are presented in Figure 1.

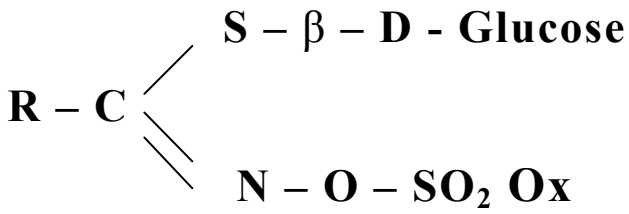


Fig 1. General Formula of Glucosinolates

Where "R" is an organic moiety and "X" is the cation (usually potassium). Various glucosinolates differ only in the nature of the R-group. The individual glucosinolates are designated by the chemical nature of the R-group, as proposed by Ettlinger and Dateo (1961). Although a number of glucosinolates are known, the major one

ePrint@CFTRI

present in *B.juncea*, *B.campestris* and *B.napus* are presented in Table 7.

The rapeseed species are not identical with respect to their glucosinolate contents. *Brassica juncea* (brown or oriental mustard) like other mustard seeds (e.g. *Brassica hirta* or white/yellow mustard and *Brassica nigra* or black mustard) is characterized by the presence of only one glucosinolate (sinigrin). While, the major glucosinolates in B. *campesteris* and *B. napus* are gluconapin and progoitrin, and to some extent glucobrassicanapin. The dry defatted seed meal of some common cultivars of rapeseed from Canada, Europe, and the Indian subcontinent contain high amount of glucosinolates, whereas newly bred Canadian and European cultivars, such as Tower and Candle in Canada, Bronowski in Poland and Erglu in Germany contain relatively low amount of glucosinolate (Table 8). However, rapeseed cultivars virtually free from glucosinolates are not yet available (Maheshwari et al., 1981). The intact glucosinolates are apparently free from toxicity, but on hydrolysis by the endogenous enzyme, myrosinase (thioglucoside glucohydrolase, E.C.3.2.3.1) yield undesirable and potentially toxic products such as isothiocyanates, thiocyanates, nitriles, and oxazolidinethiones (Fig 2). The enzyme comes into close contact with the glucosinolates during the crushing of seeds (Youngs and Wetter, 1967; VanEtten et al., 1969; Sosulski et al., 1972; Slinger, 1977).

ePrint@CFTRI

Table7. Glucosinolates in Rapeseed/Mustard

Oilseed Species	Glucosinolate	Organic radical (R)
B. napus	Gluconapin	3-Butenyl
	Progoitrin	2-Hydroxy-3-butenyl-
	Glucobrassicanapin	4-Pentenyl
	Gluconasturiin	2-Phenylethyl
	Glucoiberin	3-Methylsulfinylpropyl
	Sinalbin	p-Hydroxybenzyl
B. campestris	Gluconapin	3-Butenyl
	Progoityrin	2-Hydroxy-3-butenyl-
	Glucobrassicanapin	4-Pentenyl
	Glucoalyssin	5- Methylsulfinylpentyl
	Glucoraphanin	4- Methylsulfinylbutyl
B. juncea	Sinigrin	2- Propenyl

Source: Maheshwari et al. (1981)

ePrint@CFTRI

Table 8.Glucosinolate Content of Defatted Meal of some Selected Cultivar of Rapeseed

Rapeseed Species and Cultivar		Total Glucosinolate Content(mg/g)
B.napus	Bronowski(Polish cultivar)	0.94
	Erglu(German cultivar)	0.85
	Lesira(German cultivar)	16.70
	Target(Canadian cultivar)	9.50
	Tower(Canadian cultivar)	1.29
B.campestris	Candle (Canadian cultivar)	1.1
	Dichotoma (Indian cultivar)	9.70
	T-g, Toria (Indian cultivar)	17.80
	T-42, Yellow arson (Indian Cultivar)	6.06
B.juncea	T-63, Brown mustard (Indian cultivar)	15.10

Source: Maheshwari et al. (1981)

ePrint@CFTRI

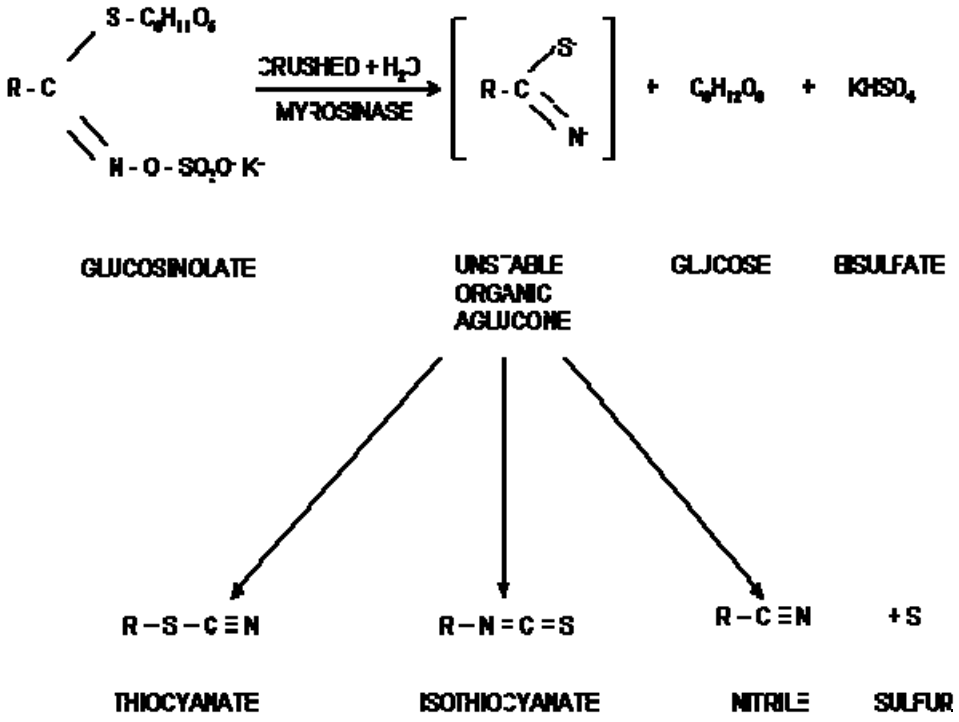


Fig 2.Enzymatic Hydrolysis of Glucosinolates

Source: Maheshwari et al. (1981)

ePrint@CFTRI

These hydrolysis products of glucosinolates when ingested by non-ruminants produce various manifestation of toxicity including depressed growth and loss in weight, loss of reproductive potential, enlarged thyroids and kidney and death in rats and other experimental animals (Downey et al., 1969; Srivastava and Hill, 1976; Srivastava et al., 1976; Maheshwari et al., 1981). Cattle and other ruminants are not sensitive to the goitrogenic factors of rapeseed meal because the toxic oxazolidinethiones are partly destroyed in the rumen of the animal (Rutkowski, 1971). However it is reported that the rapeseed meal is less palatable and less readily digested than many of other high-protein oilseed meal. Taste and actual feeding needs are the major factors that limit the dosage of rapeseed meal in the feeding of ruminants. Therefore, a small portion of the rapeseed meal is used as animal feed after diluting with other meal and the major portion is used as fertilizer. No rapeseed preparation is currently used in food for human nutrition (Maheshwari et al., 1981).

The end product of the glucosinolate-myrosinase reaction is determined by the physical, chemical and environmental conditions in which the hydrolysis reaction takes place. Shofran et al. (1998) have reported that sinigrin is hydrolyzed by thioglucoside (myrosinase) producing 1-cyano-2,3-epiopropane (CETP), allylcyanide (AC), allyl isothiocyanates (AITC), and allyl thiocyanate (ATC) (Fig 3). Mustard seed glucosinolate contain 93% sinigrin (Shahidi and Gabon, 1990). Glucobrassicin (an indole glucosinolate) hydrolyzes enzymatically to yield 3, 3`-indolylmethane, 3-indolemethanol. Isothiocyanates have a hydroxyl group at position-2, cyclise, producing oxazolidine-2-thiones (Labague et al., 1991).

ePrint@CFTRI

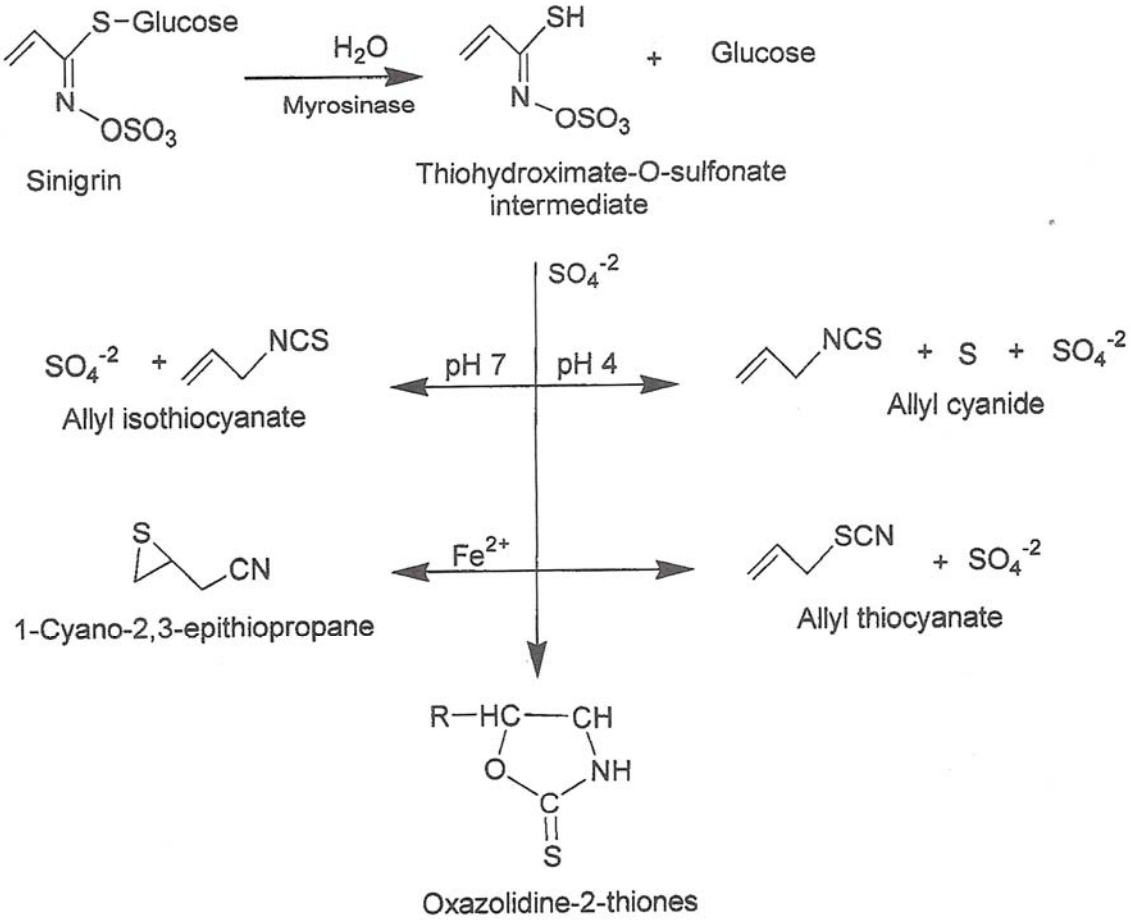


Fig 3.Myrosinase-Glucosinolate Pathway of Sinigrin Hydrolysis

Source: Whitmore and Naidu (2000)

ePrint@CFTRI

Goitrin, which is produced from 2-hydroxy-but-2-enylglucosinolate is an example of this enzymatic process. Allyl, benzyl, and 4-methylthio-butylglucosionolates are enzymatically degraded to thiocyanates. Many mechanisms have been proposed in thiocyanate formation and reported (Mieth et al., 1983a; Fenwick et al., 1983; Whitmore and Naidu, 2000).

The conditions such as pH, temperature and moisture affect the production of glucosinolate compounds. AC production proceeds at pH 4.0, whereas AITC is produced at pH 7.0, and nitrile and elemental sulfur production occurs at lower pH (Bones and Rossiter, 1996). The levels of AITC depend on the grade and cultivar of mustard/rapeseed seeds (Mayton, 1996).

A number of methods have been developed for estimating glucose- inolates, all of which are based on estimation of intact glucosinolates or the hydrolysis products. A few of the method are listed in Table 9.

The hydrolysis products of glucosinolates have been shown to have harmful effect on animals. The high content of glucosinolates and their derivatives have been shown to decrease growth, enlarge liver, kidney, thyroid and adrenal glands (VanEtten et al., 1969; Fenwick and Curtis, 1980; Nishe and Daxenbicher, 1980; Fenwick et al., 1983). These compounds are goitrogenic in nature. The thiocyanate behaves as iodine competitor while oxazolidinethione acts by interfering with thyroxin synthesis (Fenwick et al., 1983; Mithen et al, 2000; Johnson, 2002).

ePrint@CFTRI

Table 9. Methods of Glucosinolate Estimation

Intact Glucosinolate	
1. GC-HPLC	Underhill and Kirkland (1971)
2. HPLC	Helboe et al. (1980); Karcher et al. (1999); Arguello et al. (1999); Bjorkqvist and Hase (1988)
3. GLC	Olsson et al. (1976)
Breakdown Products	
A. Isothiocyanates and Oxazolidinethione	
-GLC	Youngs and Wetter (1976); Daxenbichler and VanEtten (1977)
-Spectrophotometric	Wetter and Youngs (1976); Appleqvist and Josefsson (1967)
-Titration	Anon (1980)
-GC-MS	Spencer and Daxenbichler (1980)
-HPLC	Maheshwari et al. (1979); Mullin (1978)
B. Glucose	Lein and Schőn (1969); VanEtten and Daxenbichler (1977), Olsson et al. (1976)
C. Sulphate	McGhee et al. (1965); Croft (1979)

ePrint@CFTRI

Phytates

The second major anti-nutritional factor in mustard and rapeseed is phytic acid. Phytic acid has 12 replaceable protons, allowing it to complex with multivalent cations and positively charged proteins and thus can be found in many forms. Phytate is the calcium salt of phytic acid and phytin is the calcium/magnesium salt of phytic acid. Phytates have been found to be present both in the cotyledons and in the embryotic tissue. All of these forms have been used interchangeably in most of literature linked to phytic acid systems. Phytic acid is myoinositol 1, 2, 3, 4, 5, 6-hexakis-dihydrogen phosphate, and its structure (Fig 4) suggested by Anderson (1914) is generally accepted (Thompson, 1990).Complete hydrolysis of phytic acid results in inositol and organic phosphates (Oatway et al., 2001). Phytic acid is the primary phosphorous (P) and myoinisitol reserve in most seeds and usually account for 60-90% of the total P. Phytic acid typically exists in rapeseed as salts of Ca, Mg, and K (Mills and Chong, 1977; Yiu et al., 1982). Phytic acid levels range between 2.0-4.0% in the whole rapeseed, 2.0-5.0% in defatted meals, 5.0-7.5% in protein concentrate and 1.0-9.8% in protein isolates depending on the method of protein isolation have been reported (Shah et al., 1976; Gillberg and Tornell, 1976a; Gillberg and Tornell, 1976b; Nwokolo and Bragg, 1977; Jones, 1979; Liu et al., 1982; Thompson et al., 1982b; Reddy et al., 1982; Blaicher et al., 1983; Serranio and Thompson, 1984; Thompson and Cho, 1984a; Thompson and Cho, 1984b; Thompson, 1987; Tzeng et al., 1988a; Tzeng et al., 1988b; Tzeng et al., 1990). These values are generally higher than those reported for many legumes and other oilseed (Cheryan, 1980; Reddy et al., 1982).

ePrint@CFTRI

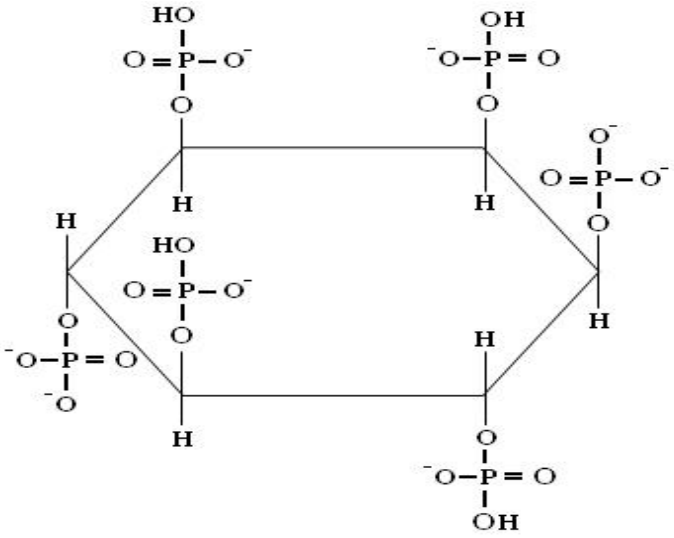


Fig 4.Structure of Phytic Acid

There are 12 replaceable protons in molecule; six are strongly dissociated with pk_a of about 1.8, two are weak acid fractions with pk_a of 6.3 and four are freely dissociated with a pk_a of 9.7 and which cannot be easily determined by visual titration methods. Hence, PA is strongly negatively charged at pH values normally encountered in foods and feeds and is very reactive with other positively charged groups such as cations and proteins (Thompson, 1990).

In general, one or two phosphate group of a PA molecule (Fig 5c) or phosphate group of different PA molecules may bind with cations. A mixed salt of PA is formed when several cations complex within the same molecule. The presence of more than one cation may increase synergically the precipitation of the phytate salts as it has been observed *in vitro* with Zn and Ca and Cu (Thompson, 1993).

ePrint@CFTRI

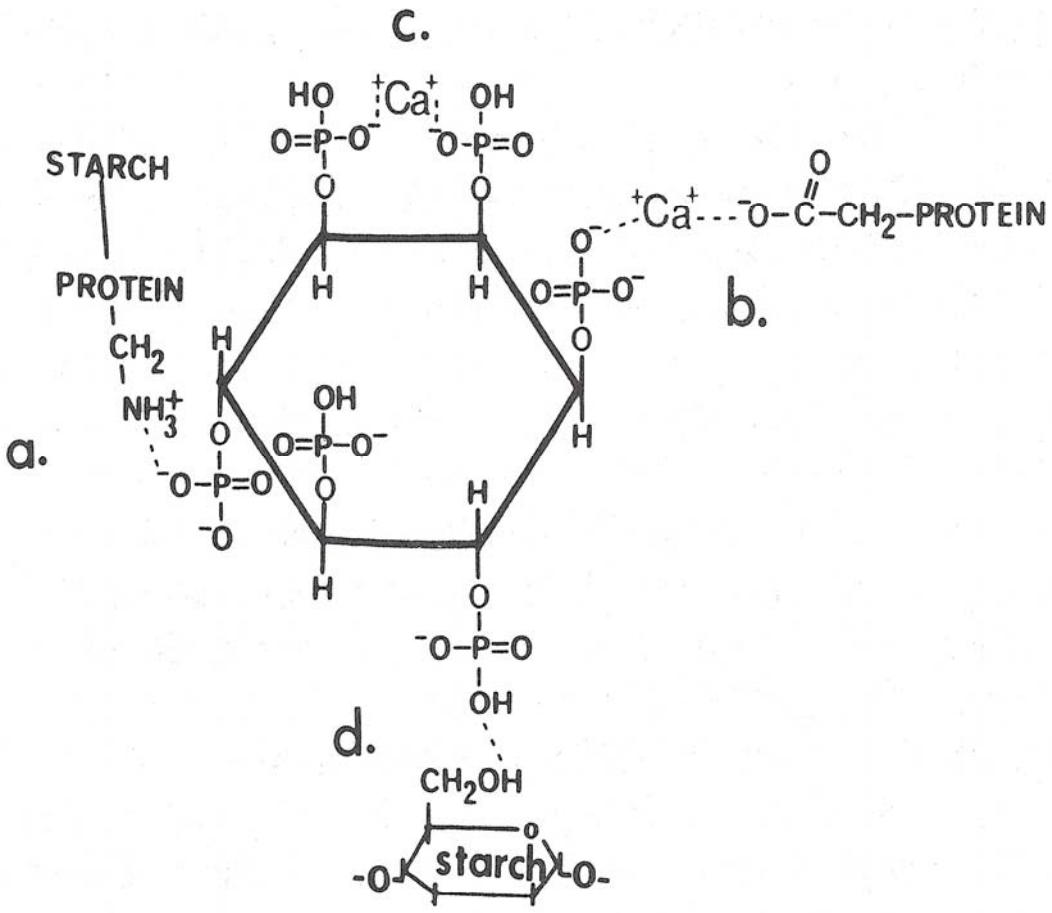


Fig 5. Possible Interactions of Phytic Acid with Minerals, Proteins, and Starch

Source: Shahidi (1990)

ePrint@CFTRI

The binding of PA with mineral ions is pH dependent and complexes of varying solubility are formed (Cheryan, 1980).Phytic acid also reacts with the protein depending on the pH. At low pH below the isoelectric point PA binds directly with the positively charged proteins as a result of electrostatic attraction (Fig 5a). Both the PA and proteins are negatively charged at intermediate pH above the isoelectric point of proteins. Therefore, PA primarily binds with the proteins mediated by multivalent cations such as Ca and Mg (Cheryan, 1980) (Fig 5b).

At pH>10, the ternary complex may dissociate with the formation of insoluble cation-PA and soluble Na-proteinate. It may be due to the shift to the right of the equation as the Na ion concentration increases with the addition of alkali at high pH (Cheryan, 1980; Thompson and Cho, 1984a; Thompson, 1987; Thompson, 1990).

$$\text{Protein-cation-PA} + Na^{+} \rightleftharpoons \text{Protein-Na} + \text{Cation-PA}$$

If the protein and PA are linked through amino group of lysine or guanidine group of arginine, the loss of charged groups at very high pH and the inability of the proteins to bind to PA may also cause the separation of the PA from proteins (Gillberg and Tornell, 1976a). The binding of starch with PA has not been demonstrated. However, binding is theoretically possible, directly through the formation of phosphate links (Fig 5d) or indirectly (Fig5a) through its association with proteins (Thompson, 1990).

A single protein molecule may bind several molecules of cations and/or PA. Complexing of the proteins with PA, directly or

ePrint@CFTRI

through mediation by mineral ions, may alter the protein structure. This in turn may lead to decreased solubility, functionality and digestibility of the proteins. Similarly, binding of minerals with PA can reduce its solubility and availability for absorption (Thompson, 1993). Phytic acid was known to affect mineral availability in as early as the 1920s, and this was discussed in several reviews (Erdman, 1979; Cheryan, 1980; Maga, 1982; Reddy et al., 1982; Thompson, 1990). Phytic acid is known to affect bio-availability of minerals such as zinc, calcium, magnesium, phosphorus and possibly iron. Zinc appears to be the most affected by PA because it forms the most stable and insoluble complex (Erdman, 1979; Cosgrove, 1980; Morris, 1986).

Numbers of methods have been reported to remove phytates from the mustard/rapeseed meal and proteins. These techniques have been developed for the removal of PA from defatted rapeseed particularly during the preparation of protein isolates and concentrate, since they contain PA level as high as 9.8%. Approaches include the use of differential solubility between protein and PA, membrane separation, enzymatic treatment and ion-exchange resins (Gillberg and Tornell, 1976a; Gillberg and Tornell, 1976b; Gillberg, 1978; Brooks and Morr, 1982; Serranio and Thompson, 1984; Seo and Morr, 1985; Lu et al., 1987; Tzeng et al., 1988a; Tzeng et al., 1988b; Tzeng et al., 1990). Acetylation and succinylation and dialysis have been shown to be effective methods to reduce PA in rapeseed protein (Cho and Thompson, 1984; Serranio and Thompson, 1984; Thompson and Cho, 1984a; Thompson and Cho, 1984b; Thompson, 1987).

ePrint@CFTRI

Phenolics

Phenolic compounds are widely distributed in plant parts from the roots to the seeds. Some phenolic compounds are essential metabolites; others have complex structures of unknown function and may be unique to a particular plant family or genera. The common phenolic compounds present in oilseed products have six or seven basic structure. These are hydroxylated derivatives of benzoic and cinnamic acids, coumarins, falvonoids and polyphenolic tannins and lignin (Sosulski, 1979).

The predominant phenolics present in rapeseed/mustard are phenolic acids and condensed tannins. The content of phenolic acids in rapeseed flour (dehulled defatted rapeseed) is in the range of 10 to 30% higher than that found in flours obtained from other oleaginous seeds. However, the content of phenolic acids in rapeseed meal (defatted whole rapeseed) is up to five times higher that those found in soybean meals (Table 10). Therefore, phenolics are considered as important factors when canola/rapeseed meal is to be used as a source protein for human (Naczk et al., 1998).

Total Phenolics Acids

Phenolic acids in rapeseed are present in the free, esterified and insoluble-bound forms and are derivatives of benzoic and cinnamic acids. *Trans*-sinapic acid is the predominant phenolic acid found in rapeseed/canola. The total content of phenolic acids in rapeseed meal is up to 18.4 g/kg in defatted meal. In contrast, full fat rapeseed flour contains about 6.2 to12.8 g/kg total phenolics acids (Kozlowska et al., 1975; Krygier et al., 1982; Kozlowska and Zadernowski, 1983; Naczk and Shahidi, 1989; Naczk et al., 1992).

ePrint@CFTRI

Table 10.Total Content of Phenolic Acids in Some Oilseed Products

Oilseed Product	Phenolic Acids (g/kg dry basis)
Soybean Flour	0.23
Cottonseed Flour	0.57
Peanut Flour	0.63
Rapeseed/Canola Flour	6.4-12.8
Canola Meal	15.4-18.4
Soybean Meal	4.6

Source: Naczk et al. (1998)

ePrint@CFTRI

Free Phenolic Acids

Rapeseed meal contains about 2 g/kg free phenolic acids (Naczk and Shahidi, 1989; Naczk et al., 1992), whereas the content of free phenolics in rapeseed flours range from 0 to 1g/kg (Krygier et al., 1982; Kozlowska and Zadernowski, 1983). Sinapic acid constitutes from 70% to 85% of the total free phenolic acids. Rapeseed/canola products also contain small quantities of p-hydroxy-benzoic, vanillic, gentistic, protocatechuic, syringic, *p*-cumaric, *cis*- and *trans*-ferulic, caffeic and chlorogenic acids in free form (Krygier et al., 1982; Kozlowska et al., 1991).

1. Esterified Phenolic Acids

Esterified phenolic acids are the predominant fraction of phenolic acids present in rapeseed protein products as they constitute up to 80% of the total phenolic acids. Sinapic acid constituted 71-97% of the phenolic acids liberated from the soluble fraction of esterified phenolic acids in rapeseed/canola meal (Krygier et al., 1982; Naczk et al., 1998). Sinapine, the most abundant phenolic ester in rapeseed, is a bitter phenolic compound which contributes to the unpleasant and bitter flavor.

2. Insoluble-bound Phenolic Acids

Canola meals contain approximately 1g/kg insoluble-bound phenolic acids (Naczk and Shahidi, 1989). In contrast, full fat rapeseed flours contain 32-50 mg/kg insoluble-bound phenolic acids (Kozlowska and Zadernowski, 1983). Nine phenolic acids were identified in the fraction of insoluble-bound phenolic acids isolated from rapeseed flours. Sinapic acid was found to be the predominant phenolic acid and it constituted 30 to 59% of total insoluble fraction

ePrint@CFTRI

of phenolic acids in rapeseed and mustard flour (Durkee and Thivierge, 1975; Kozlowska and Zadernowski, 1983).

3. Condensed Tannins

Tannins are complex phenolic compounds having a molecular weight in the range of 500-3000 Da. They are classified as condensed or hydrolysable, based on their structure and reactivity towards hydrolytic agents, particularly acids (Naczk et al., 1998). Shahidi and Naczk (1989) found that canola meals contained 0.68-0.77% of condensed tannins. On the other hand, Mitaru et al. (1982) reported that rapeseed hulls contain 0.02-0.22% condensed tannins. According to Leung et al. (1979) leucocyanidin is a basic unit of tannins isolated from rapeseed hulls. The condensed tannins may form complexes with proteins thus lowering the nutritional values of the protein products (Naczk et al., 1996; Naczk et al., 1998).

Phenolics and Meal Quality

Phenolic compounds contribute to the dark color, bitter taste and astringency of rapeseed meals. Phenolics and their oxidized products may form complexes with the essential amino acids, enzymes and other proteins, thus lowering the nutritional value of rapeseed meal as a protein source (Kozlowska et al., 1975; Naczk and Shahidi, 1989; Naczk et al., 1992; Naczk et al., 1998). The presence of tannins have been shown to be associated with lower nutritive value and lower biological availability of macromolecules like proteins, carbohydrates, amino acids, vitamins and minerals (Deshpande et al., 1986; Makkar et al., 1987; Makkar, 1989).

ePrint@CFTRI

Hulls

The anti-nutritive hull constituents are high molecular weight polysaccharides, like hemicellulose and pentosans as well as poly-phenols like lignin and tannins that constituting 90% of fat-free dry matter (Aspinall and Jiang, 1974; Siddiqui and Wood, 1976; Siddiqui and Wood, 1977; Theander et al., 1977; Leung et al., 1979). The content of hulls in rapeseed/mustard varies from 10.5 to 20% of seed weight and 20 to 30% in defatted meal (Appleqvist and Ohlson, 1972; Theander et al., 1977; Bell, 1993; Jensen et al., 1995).

The commercial rapeseed meal contains 12.1% crude fiber, most of which is derived from hulls (Table 11). About 50% of the meal and 80% of the hull are composed of crude fiber and N-free fractions. Rapeseed hulls are high in crude fiber (67%) and in acid and neutral detergent fiber (80%), respectively. The composition of hulls differs between varieties with brown and yellow hulls (Bell and Shires, 1982).The nature of carbohydrate and the amount of lignin present in rapeseed hulls(Table 12) lowers the digestibility of hull in monogasteric animals (Bell, 1984). The hulls are indigestible or only metabolisable after predigestion in the rumen. The use of hulls in feed formulation decreases the feeding value and digestibility. This may be due to the blocking effect of anti-nutritional hull constituents on the alimentary proteins or enzymes of the gastro-intestinal tract (Mieth et al., 1983a). The hulls of mustard and rapeseed are not commonly removed in the commercial plants. The small seed size and the high oil content of the seeds make it difficult to dehull efficiently. After cleaning, the seeds are crushed by screw (expeller) pressing and followed by solvent extraction to reduce the oil content in the cake to a level of 0.5%. In India, generally the mustard/

ePrint@CFTRI

rapeseeds are crushed by Ghanis (driven by bullock or power), or expeller is used for oil extraction.

The mustard/rapeseed meal is potentially a high quality protein supplement but its use in the diets of monogasteric animals is limited by the presence of high content of indigestible fiber in the meal (Slominski et al., 1994). The low digestibility of rapeseed is a serious problem, which is partly due to the hull fraction that is high in insoluble dietary fiber (Liesle et al., 1973; Jones and Sibbald, 1979; Sarwar et al., 1981; Bjergegaard et al., 1991).

A number of processes for dehulling of rapeseed/canola have been proposed and reported. The removal of hulls can be accomplished by air-classification of the defatted meal (Tape et al., 1970; Diosady et al., 1986), by liquid cyclone fractionation after solvent extraction (Sosulski and Zadernowski, 1981) or by cracking and air classification (Jones, 1979; Schneider, 1979; Jones and Holme, 1982).

A process for the dehulling of mustard/rapeseed has also been developed by the Central Food Technological Research Institute, India. This process includes the grading of seeds to separate small seeds, conditioning of seed, drying and cracking. The cracked seeds were separated into three fraction; hulls, fines and meal by air classification. The hull fraction amounts 15-20% of the seed weight (Kantharaj Urs and Kowsalya, 1976).

ePrint@CFTRI

Table 11. Proximate Analysis of Rapeseed Meal and Hull (Dry Matter Basis)

Component (%)	Meal	Hulls
Crude Protein (N×6.25)	42.8	12-16
Crude Fiber	12.1	44
Ether Extract	4.1	3
Ash	7.0	4-5
Nitrogen-Free Extract	34	34

Source: Bell (1984)

ePrint@CFTRI

Table 12.Carbohydrate and Related Factors Found in Rapeseed Hulls

Component	Hulls (%)
Nitrogen-Free Extract	34
Crude Fiber	44
Pentosans	14.5
Cellulose	32
Sugars	3.8
Lignin	12-24
Polyphenols	6-12
Tannins	1.5

Source: Bell (1984)

ePrint@CFTRI

Improving the Quality of Rapeseed/Mustard Protein for Human Use

In view of world-wide protein shortage, oilseeds are becoming of increasing importance as a source of edible proteins (Altschul, 1974). Rapeseed, which is a major oilseed crop of temperate zones, has so far found little application in the production of protein. The seed contain about 42% oil, which is widely used as edible oil. The defatted meal contains up to 40% protein which is used in animal feeds or as an organic fertilizer. The protein has a well balanced amino acid composition (Ohlson and Anjou, 1979; Sarwar et al., 1984) and compare favorably with soy proteins and the FAO pattern of essential amino acid requirement of human adults. Mustard/ rapeseed proteins are rich in lysine and methionine, which are limiting essential amino acids in most of the cereals and other vegetable proteins (El-Nockrashy et al., 1975a; El-Nockrashy et al., 1975b). Therefore, rapeseed/mustard protein may be considered as a potential source of food protein. In addition, rapeseed/mustard protein products have satisfactory functional properties for use in various formulations for human consumption. Currently, considerable interest has been focused on rapeseed meal as a source of food proteins, not only because of its high amount of high quality proteins, but also of its low cost and availability.

The use of rapeseed meal as a protein source in livestock and human food is limited due to the presence of glucosinolates and other undesirable compounds such as phytates, phenolics and hull. These toxic and anti-nutritional components have to be removed completely before rapeseed protein could be used for human consumption (Tzeng et al., 1988a). The composition of rapeseed has been significantly altered by the Canadian breeders who have

ePrint@CFTRI

developed new varieties of rapeseed, known as "canola". Despite developing canola varieties with low glucosinolate and erucic acid content, several major problems to be solved prior to use of canola meal as a protein source in food products are, the presence of glucosinolate (1-3 mg/g) which is still too high for incorporation into food products (Naczk et al., 1998); high amount of phytic acid and phenolics in rapeseed meal (Naczk et al., 1998); non-adaptability of these varieties to agro-climatic condition in some countries, especially India, that results late maturity and poor seed sets (TERIvision, 2001) and presence of unidentified anti-nutritional factors in new low glucosinolate varieties (Josefsson and Munck, 1972; Srivastava and Hill, 1976; Maheshwari et al., 1981).

Because of the superior quality of rapeseed/mustard protein for human nutrition and presence of anti-nutritional constituents in meal along with protein, over the last three decade many methods have been developed to upgrade its meal. Such processes are generally termed "detoxification" and involve chemical, microbial or physical treatments, alone or in combination. Some of these methods will be discussed below:

Detoxification of Rapeseed/Mustard

Removal of glucosinolate and/or their decomposition products from rapeseed and canola products are generally termed as "detoxification". The methods of detoxification involve chemical, microbiological or physical treatment (Fenwick et al., 1986) and have been divided into five categories (Maheshwari et al., 1981):

1. Inactivation of the enzyme myrosinase. At present it is practiced in commercial processing of canola by using moist heating

ePrint@CFTRI

(Reynolds and Youngs, 1964; Appleqvist and Josefsson, 1967), immersion of seeds in boiling water (Belzile et al., 1963; Eapen et al., 1968) and microwave heating (Maheshwari et al., 1981). Presence of moisture was found to be an essential factor in this method. If myrosinase was inactivated beforehand, the meal would presumably be non-toxic because unhydrolyzed glucosinolates are not goitrogenic. However, this method is of limited value due to the possible presence of myrosinase in the gastro-intestinal tract and in bacteria (Woyewoda et al. 1978; Maheshwari et al., 1981). It has been shown that the bacterial microflora of human colon and other mammals can express myrosinase activity. Significant quantities of isothiocyanate metabolite are excreted in the urine of healthy human volunteer after eating *Brassica* vegetable, even when myrosinase has been completely inactivated by cooking (Johnson, 2002).

2. Eliminating glucosinolates from rapeseed and canola by biotechnological methods. Rapeseed breeding programs launched in the early 1960`s have produced some varieties of rapeseed with low glucosinolate contents, such as Tower and Candle in Canada, Bronowski in Poland and Erglu in Germany. However, defatted meals prepared from the low glucosinolate varieties still contain appreciable amounts of glucosinolates which may restrict their use in animal feed and human nutrition. Rapeseed varieties virtually free of glucosinolates still are not available. Most of the rapeseeds cultivated currently in the East are of the high glucosinolate type. Also, the studies have shown that low glucosinolate cultivars of rapeseed, Bronowski and Tower are not so toxic but may contain other anti-nutritional factors and some high molecular weight compounds that exert a detrimental effect on the nutritive value of

ePrint@CFTRI

the rapeseed meal (Josefsson and Munck, 1972; Srivastava and Hill, 1976).

3. Potentiation of the enzyme and removal of decomposition products. In these processes, in addition to the endogenous myrosinase present in the seed more myrosinase may be added to the system to liberate glucosinolate and then the breakdown products will be removed (Goering, 1963; Vaccarino et al, 1976; Mukherjee et al., 1976). This method may have the disadvantage of leaving the organic aglucons in the oil. A process for preparation of bland protein from dehulled defatted mustard meal was developed at CFTRI, Mysore (Kantharaj Urs and Kowsalya, 1976). In this process, the dehulled defatted seed in the form of flour was mixed with water (1:3 w/v) and the pH was adjusted to 8.2. A paste of ground, cold defatted mustard seed was added as enzyme source and the glucosinolate hydrolysis was carried out at room temperature for 4-5 h. The allyl isothiocyanate was removed by steam distillation. The aqueous slurry was cooled and its pH was adjusted to 5.0. The solid matter which was collected by centrifugation was washed with water to remove the bitter compounds and dried.

4. Destruction of glucosinolate and their breakdown products. This may be achieved by the use of heat, chemicals and micro-organisms (Bell et al., 1970; Kirk et al., 1971; Anderson et al., 1975; Youngs and Perlin, 1976; Bell et al., 1981; Mieth et al., 1983b). Methods used for destruction of glucosinolates are not satisfactory. These methods result in loss of nutritive value of the protein and are not economical for large scale industrial applications. The products formed by chemical agents may be more toxic than the glucosinolates or its hydrolyzed products (Maheshwari et al., 1981).

ePrint@CFTRI

5. Removal of glucosinolates and the hydrolysis products. This method includes diffusion extraction of intact glucosinolates or their hydrolytic products (Ballester et al., 1970; Tape et al., 1970; Sims, 1971; Bhatty et al., 1972; Kozlowska et al., 1972; Sosulski et al., 1972; Ballester et al., 1973; Afzalpurkar et al., 1974; McFarlane et al., 1976; Jones and Holme, 1979; McGregor, 1983; VanMagan, 1983). In some of the studies, cold or warm water or buffers has been employed to extract glucosinolate and their hydrolytic products from crushed rapeseed/mustard flour or defatted meal (Belzile and Bell, 1966; Belzile et al., 1963). Ultrafiltration techniques have also been suggested by Bockelmann et al. (1977) for removal of glucosinolates and their hydrolysis products.

A number of methods have been reported in the literature for detoxification of rapeseed/mustard. These method includes using chemicals, sodium Hexamethaphosphate extraction and isoelectric precipitation; ultrafiltration; methanol-ammonia-water extraction to reduce glucosinolates and phenolics; two phase extraction with methanol-ammonia-water and hexane; sodium hexamethaphosphate extraction followed by activated carbon treatment, ultrafiltration, diafiltration and ion-exchange; aqueous sodium hexamethaphosphate or sodium hydroxide extraction, ultrafiltration, isoelectric precipitation and diafiltration; alkaline extraction, isoelectric precipitation, ultra-filtration followed by diafiltration; alkaline extraction and isoelectric precipitation (Liu et al., 1982; Thompson et al., 1982b; Diosady et al., 1984; Diosady et al., 1987; Shahidi et al.,1988; Tzeng et al.,1988a; Tzeng et al.,1988b; Tzeng et al.,1990; Zhou et al., 1990; Klockman et al., 1997). Schwenke et al. (1990) have reported that treatment of the rapeseed flours with citric acid or ammonium carbonates lower the sinapine, glucosinolates and their hydrolyzed products. A post

ePrint@CFTRI

treatment with alcohol/ammonia/water resulted in total elimination of vinyl oxazolidinethione, nitriles and isothiocyanates. The protein micellar mass (PMM) procedure has been used to isolate protein from canola by Ismond and Welsh (1992). Protein isolate is also prepared by alkaline extraction and treating the extract with NaCl, SDS and combined treatments to prevent the phenolics interaction with the proteins (Xu and Diosady, 2002).

Processes for the preparation of protein isolate from mustard, rapeseed and canola by different treatments to reduce anti-nutritional constituents have been developed and patented (Cameron and Myers, 1982; Cameron and Myers, 1983; Diosady et al., 1989; Diosady et al., 2003; Murray and Westdal, 2005; Hiron et al., 2005; Barker et al., 2005; Diosady et al., 2005).

None of the above procedures completely eliminates all the anti-nutritional and toxic constituents. Therefore, at present the rapeseed/mustard meal finds limited uses as a source of protein for human food or animal feeds. So it is obvious that the only means of using rapeseed/mustard meal as a source of protein for human consumption lies with the development of suitable method to remove or eliminate the anti-nutritional and toxic constituents from the meal.

ePrint@CFTRI

Scope and Objectives of the Present Investigation

Rapeseed/mustard is an important oilseed crop in the world in general and India in particular. Most of the seed are used for oil production and the cake obtained contains 35-45% protein, is being used for animal feed or fertilizer. The presence of high glucosinolate, phenolics, phytate and fiber in cake restricts the widespread use of Indian rapeseed/mustard cake as animal feed.

In India, rapeseed/mustard oil is mainly used for edible purposes and the cake, rich in protein is the by-product of oil seed industry, primarily is used for feeding animals and poultry due to its high protein content. Traditionally, rapeseed/mustard is processed by crushing the whole seed without any dehulling and the cake obtained contains high amounts of glucosinolates and fibre which are not desirable for animal feed leading to low productivity. The high levels of glucosinolates in Indian rapeseed/mustard varieties also lead to nutritional disorders and toxicity in human beings as well as animals. Hence, the presence of these anti-nutritional constituents has limited the use of cake in food/feed purposes. The cake attracts a very low price in the international market because it does not meet the desired international standards due to the presence of high amounts of glucosinolates. Cake has to be processed to remove these constituents and keep them within the acceptable levels.

The meal proteins are rich in lysine and contain adequate amount of methionine, which are the limiting amino acids in most of the cereal and oilseed proteins. The meal protein has a well balanced amino acid composition. It has been shown that the quality of protein is similar to casein and superior to the proteins of many other vegetable sources such as soy, pea and wheat; thus it may be considered as a

ePrint@CFTRI

potential source of food protein. However, the use of rapeseed/mustard meal as a protein source in food products is limited by the presence of glucosinolates, phytates, phenolics and fiber. Glucosinolates on hydrolysis by the enzyme myrosinase forms toxic compounds that interfere with thyroid function. These toxic compounds are known to reduce the biological value, growth and damage the liver and kidney. Phytates are strong chelating agents that affect the utilization of polyvalent metal ions, especially zinc and iron. Phenolic compound have a bitter flavor and cause dark colors in the protein products. The hull constitutes approximately 30% of the oil free meal, is high in indigestible fiber. These toxic and anti nutritional compounds have to be removed before the protein could be used for human consumption.

In order to eliminate these toxic and anti nutritional factors, number of methods have been reported in the literature. These being: steaming, toasting, wet heat processing, water washing, chemical treatment, membrane processing, dialysis, ultra filtration, diafilteration, ion exchange, protein micellar mass (PMM) procedure and microbial degradation. The limitations of these methods are: loss of protein, poor functionality, high processing cost, and low yield of protein. At present commercially viable processes for the production of food grade rapeseed/mustard protein are not available. Therefore, in the present investigation studies were focused on:

1. Dehulling, size reduction and air classification to remove the hulls from seed;
2. Size reduction and separation of commercial cake to reduce the hull content;

ePrint@CFTRI

3. Standardize the conditions to eliminate the anti nutritional and toxic components and to isolate the protein from rapeseed/mustard meal and cake;
4. To study the effect of processing on various physicochemical and functional properties of proteins;
5. To evaluate the nutritional quality of protein products; and
6. To develop protein rich spaghetti product by supplementation with mustard protein isolate

ePrint@CFTRI

Materials

Mustard Seeds

Mustard seeds of the variety T-59 were purchased from Karnataka state seeds corporation limited, Karnataka, India. This variety was used for preparation of dehulled defatted flour. A commercially available variety purchased from local market of Mysore, India was used for preparation of commercial mustard cake.

Semolina

Semolina for spaghetti making was purchased from local market of Mysore, India.

Chemicals

The different chemicals used were from following sources:

Amino acid standards and PITC (Phenylisothiocyanate) were from Pierce H.; low range molecular weight markers were from Genie, Bangalore, India; Acrylamide, bis-acrylamide, Ammonium Persulphate, TEMED, β-mercaptoethanol, Coomassie Brilliant Blue, Thioglucosidase (EC 3.2.3.1), Trypsin, Panceratin, Pepsin, Tannic acid, Phytic acid (from corn), Nα-Benzoyl-DL-Arginine-ρ-Nitroanilide (BAPNA), mono Є-N-dinitrophenyl-lysine hydrochloride monohydrate (DNP-Lysine) were from Sigma, USA; 1-fluro-2,4-dinitrobenzen (FDNB), DL-Tryptophan, Ascorbic acid were from E-Merck, Germany; Alcalase and Termamyl was from NOVO Industries, Denmark; Ninhydrine from S.D.Fine-Chemicals limited, Mumbai, India; Trinitrobenzenesulfoinic Acid (TNBS) from Eastman Kodak Co., USA; and Activated carbon was from Nice Chemicals Pvt, Ltd. Cochin India. All other chemical used were from analytical reagent grade (AR).

ePrint@CFTRI

Methods

Preparation of Dehulled Defatted Flour

The dehulled defatted mustard meal was prepared according to the method developed at CFTRI and described by Kantharaj Urs and Kowsalya (1976). The mustard seeds were graded. The graded seeds were equilibrated with the addition of 10% moisture and dried at 60°C to a moisture level of 6%. The dried seeds were passed through plate mill to split into two halves and air classified to remove hulls. The dehulled kernels were passed through sieve to remove fines. The dehulled kernels (10 kg) were flaked to 0.15 mm thickness using flaking rolls (kvarnmaskiner, Malmo, Type J. No. 6725) and extracted with hexane in the ratio of 1:5 (w/v) using stainless steel columns. The extraction was repeated 4-5 times with a soaking time of 12 h each. The fat content of flakes was 1%. The flakes were air dried (~28°C) and powdered and passed through 223 µm sieve and stored at 4°C till the analysis (Fig 6).

Preparation of Commercial Mustard Cake

The mustard seeds were equilibrated with addition of 10% moisture and kept for 30 min at room temperature. The seeds were crushed by mechanical pressing using power Ghani (Fig 7) to extract the oil.

ePrint@CFTRI

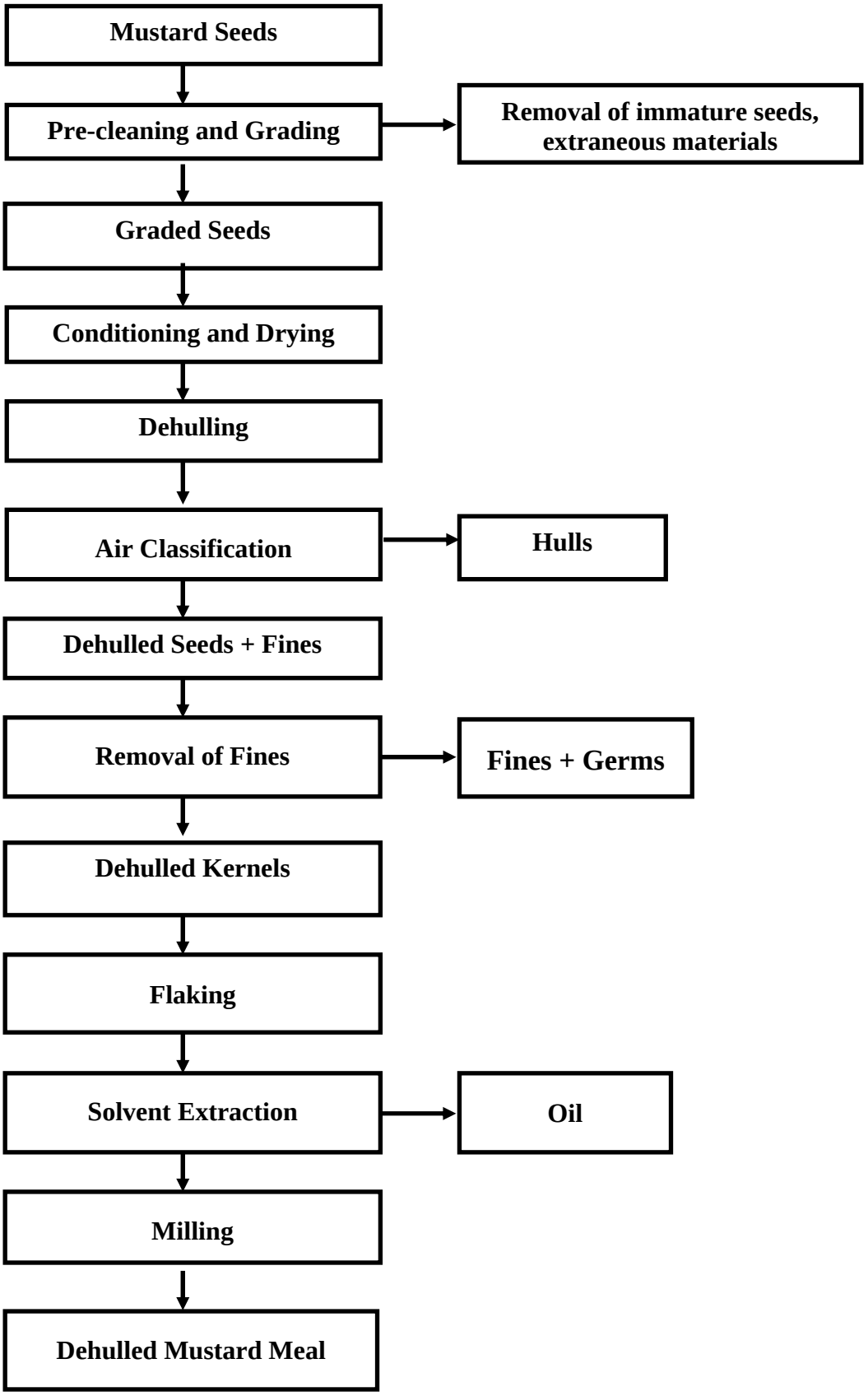


Fig 6. Flow Sheet for the Preparation of Dehulled Mustard Flour

ePrint@CFTRI

Fig 7. Power Ghani

ePrint@CFTRI

Fractionation of Mustard Cake

The cake after oil extraction was dried in a cross flow drier to a moisture level of 6% and extracted with hexane in a ratio of 1:5 (w/v) using stainless steel column. The extraction was repeated 4-5 times with a soaking time of 12 h each. The fat content of cake meal was less than 2%. The cake meal was air dried (~28°C) and fractionated with a Quadromat mill (Quadromat® Senior, Brabender) (Fig 8) into three fractions viz. low hull high protein fine, low hull high protein coarse, and high hull fraction (Fig 9).

Isolation of Protein from Mustard Flour/ Commercial Cake

Protein isolate/concentrate was prepared according to the method developed and standardized in our laboratory (Fig 10). Defatted mustard flour 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°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 min, cooled and centrifuged at 5000 rpm to separate protein. The precipitate was dispersed in water in a ratio of 1:10 (w/v) and centrifuged. The washing process was repeated again. The wet protein isolate was dispersed in water and neutralized with HCl/NaOH.

ePrint@CFTRI

Fig 8. Quadromat Mill

ePrint@CFTRI

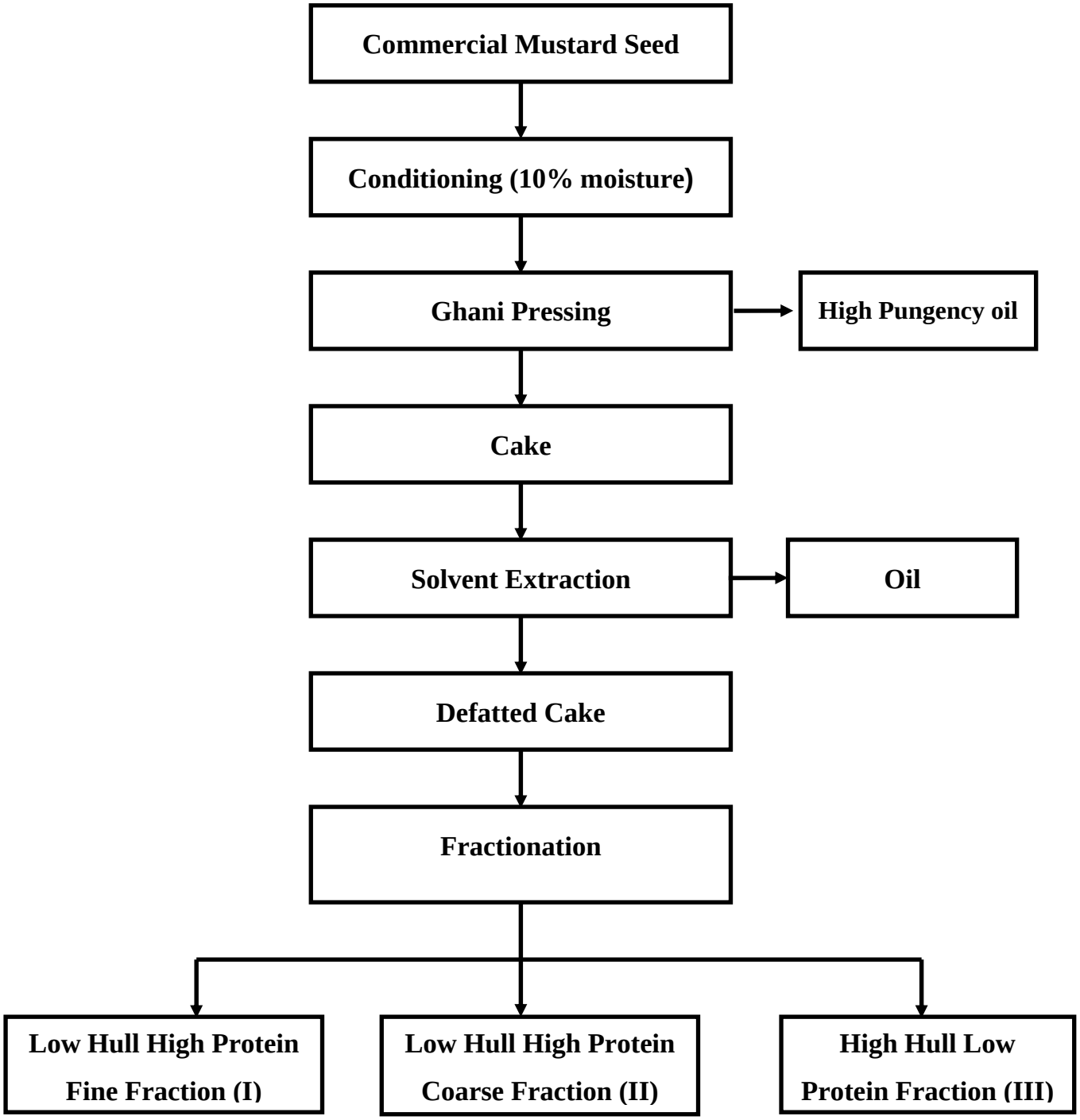


Fig 9.Flow Diagram for the Preparation and Fractionation of Commercial Cake

ePrint@CFTRI

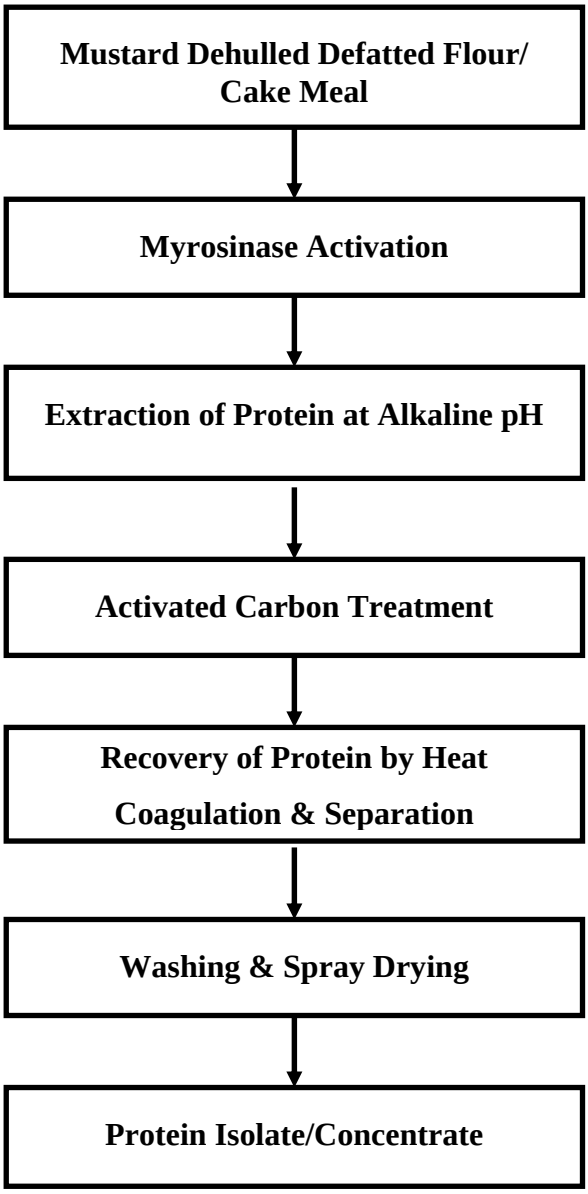


Fig 10. Flow Sheet for the Preparation of Mustard Protein Isolate/Concentrate

ePrint@CFTRI

The dispersion with a solid content of 20% was spray dried (Bowen Engineering, Inc, New Jersey, USA). The inlet temperature of 150 ± 5°C was used. Same method was used for extraction of protein from low hull and high protein fraction (mixture of fraction I and II) of cake meal.

Preparation of Protein Hydrolysate from Dehulled Defatted Flour/Commercial Cake Meal

The protein hydrolysate was prepared by the method given in Figure 11. The coagulated protein after washing (Fig 10) 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 (Fig 11).

Isolation and Recovery of Proteins by Different Methods

To study the effect of different methods on the recovery of protein, yield and removal of anti-nutritional constituents of protein, the alternative methods namely (a) isoelectric precipitation, (b) alcoholic precipitation and (c) ultrafiltration have been used.

ePrint@CFTRI

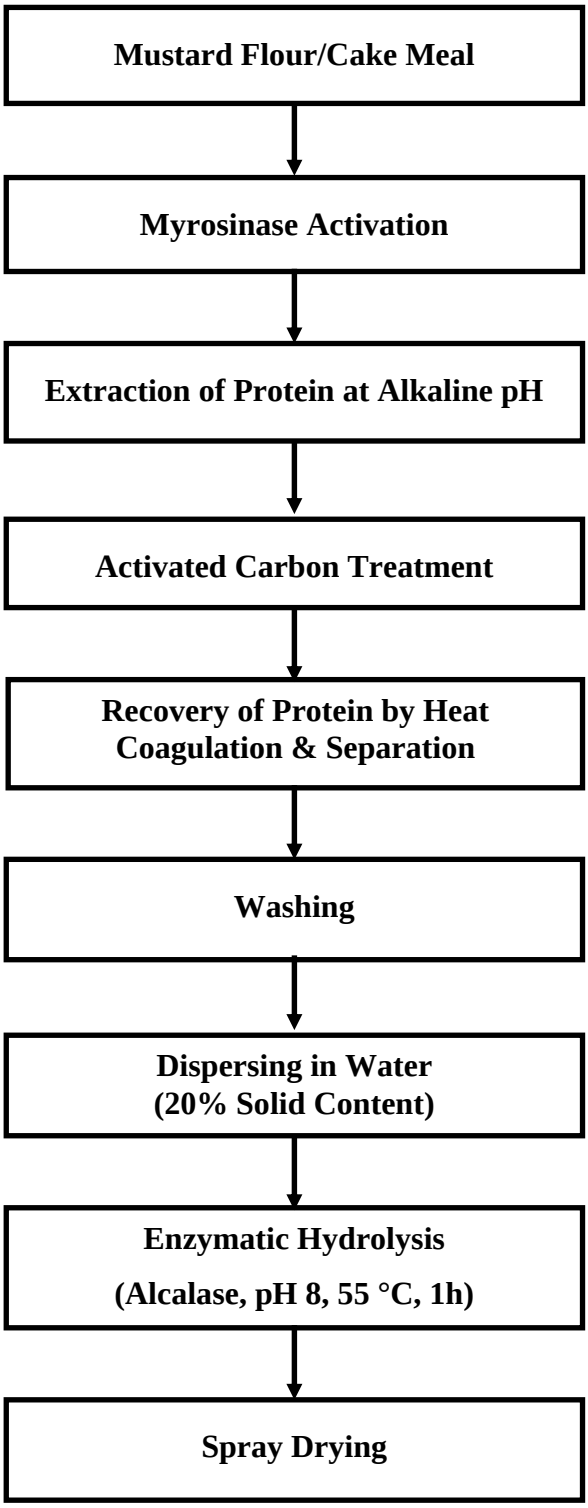


Fig 11. Flow Sheet for the Preparation of Protein Hydrolysate from Dehulled Mustard Flour/Cake Meal

ePrint@CFTRI

(a) Recovery of Proteins by Isoelectric Precipitation

In isoelectric precipitation, two isoelectric pH (7.8 and 3.8) were used respectively. The solution after treating with activated carbon was adjusted to first isoelectric point (pH 7.8), using 1 N NaOH and the solution was centrifuged and precipitated protein was recovered. The pH of supernatant re-adjusted to second isoelectric point (pH 3.8) using 1N HCl, and solution was centrifuged. The two precipitated protein were combined, washed and spray dried.

(b) Recovery of Proteins by Alcoholic Precipitation

In alcoholic precipitation method, the protein solution after treatment with activated carbon was mixed with ethyl alcohol (30% of protein solution) and precipitated proteins were separated by centrifugation, washed and spray dried.

(c) Recovery of Proteins by Ultrafiltration

In ultrafiltration method, the protein solution after activated carbon treatment was passed through ultrafiltration unit (Watson Marlow, Model No.623S, Falmouth, Cornwall, TR 11 4Ru, England). Generally, a built-in peristaltic pump (Bradel Pump) drew solution from a sample container, pumped it through a Polysulfone membrane cartridge (Sartocon slice, 30S 1463901 E-SG with a nominal molecular weight cut-off of 10 KD). Pressure in the cartridge was controlled by a back-pressure valve at the outlet. The retentate was returned to the sample container (Fig 12).

The sample flows continuously through ultrafiltration unit till its volume decrease to 1:10 original volume. The concentrated sample was diluted with water to 20% of solid material and spray dried.

ePrint@CFTRI

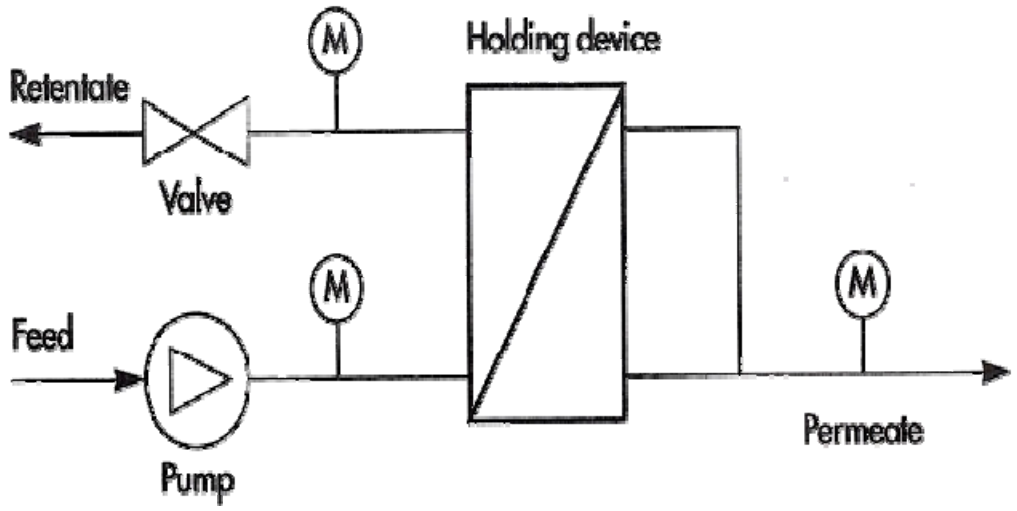


Fig 12. Cross Flow Diagram of Ultrafiltration Unit

Production of Spaghetti

Optimum of semolina water absorption was determined subjectively after several trails. Optimum water absorption depends on the water to mix uniformly with semolina and to produce a uniform granular mixture which produces spaghetti strands without any specks. Care was taken not to add excess water which would otherwise adversely affect the extrusion and drying of the extruded dough. The following steps were followed for the production of spaghetti:

Pre-Mixing: Semolina was mixed with pre-calculated amount of distilled water (40°C) in a Hobart mixer (Model N-50, Ontario, Canada) at a beater speed 1 (61 rpm) for 5 min to make 500 g dough. The pre-mixing result a more uniform distribution of water and help to produce spaghetti with uniform appearance without specks.

ePrint@CFTRI

Mixing and kneading: The pre-mixed mixture of semolina and water was transferred to a laboratory pasta machine (La Monferrina, Model Dolly; Via A.Vespucci, 38/40-14100 Asti, Italy) and mixed for 10 min (Fig 13). Kneading process imparts continuous pressure on the dough so that the dough is compressed just enough for it to withstand the extrusion and shaping process.

Extrusion: The dough was extruded using single screw laboratory pasta machine (Fig 13). The temperature of extruded dough was around 37-40°C. A 36-strand, 1.7 mm diameter die was used to shape the dough as spaghetti strands.

Drying: The extruded raw spaghetti was hung on wooden sticks and kept in drier. High temperature short time (HTST) drying system was used maintaining the temperature at 85°C. The relative humidity of the chamber was progressively decreased from 90% to 65% during a drying period of 5h. The dried spaghetti was cooled and packed in polyethylene bags for further experiments. An interval of 10 days at room temperature was given to stabilize the dried samples prior to conducting the cooking studies.

Preparation of Enriched Spaghetti Samples

Enriched spaghetti formulations were made by supplementing with 2.5%, 5% and 10% mustard protein isolate. These samples were used to study the effect of enrichment of semolina with mustard protein isolate on the rheological properties, chemical composition, cooking quality, nutritional quality and sensory evaluation. Spaghetti without enrichment was used for comparison as control.

ePrint@CFTRI

Fig 13. Laboratory Pasta Machine

ePrint@CFTRI

Analytical Procedures

Chemical Composition

The mustard seed, meal, cake, different fractions of cake and spaghetti samples were analyzed by standard methods 934.01, 988.05, 920.39, 942.05 and 962.09 (AOAC, 2000) for moisture, protein (N×6.25), fat, ash and crude fibre, respectively.

Glucosinolates

The isothiocyanates and oxazolidinethiones, the hydrolyzed products of glucosinolates, were estimated as described by Wetter and Youngs (1976).

Rapeseed flour (100 mg) is weighed into a 4 ml screw-cap vial with a Teflon Cap liner. One milliliter of the phosphate-citrate buffer containing 3mg of thioglucoside glucohydrolase (Myrosinase) and exactly 2.5 ml of methylene chloride was added to the vial. The capped vial containing one glass bead is shaken on an oscillating shaker for 2h at room temperature.

After completion of the enzyme incubation, the emulsion is broken by centrifugation at 3000 rpm. A clear methylene chloride layer is essential, as trace of aqueous extract will introduce large errors into the assay. The total isothiocyanate plus oxazolidine-2-thione content was determined by adding 50 µl of the methylene chloride extract to 3 ml of 20% ammoniacal ethanol (1 part concentrated NH_4OH to 4 parts anhydrous ethanol) and heating for 2h at 50°C in a water bath. The reaction is carried out in a culture tube (16×125mm) equipped with a screw cap. After cooling, the optical density was determined at 235, 245 and 255 nm. The blank was made up of 50 µl of methylene

ePrint@CFTRI

chloride in 3 ml of 20% ammoniacal ethanol. The corrected optical density was obtained as follows:

$$OD_{245corr} = OD_{245} - ½ (OD_{235} + OD_{255})$$

The total isothiocyanates content was expressed as mg 3-butenyl isothiocyanate per g of flour.

mg of 3-butenyl isothiocyanates per g flour = $OD_{245corr} \times [28.55]$

5-vinyloxazolidine-2-thione content was determined by following the step outlined above, except that 95% ethanol was substituted for the ammoniacal solution. The amount in the meal was expressed as mg of 5-vinyloxazolidine-2-thione per g of meal.

mg of 5-vinyloxazolidine-2-thione per g of meal = $OD_{245corr} \times [22.1]$

Phenolics

Total phenolics were estimated as tannic acid equivalents according to the method described by Deshpande et al. (1986) with slight modification.

0 to 100 ml aliquots of the standard tannic acid solution was added into 100 ml volumetric flask containing 75 ml of water. Into each of the volumetric flasks 5 ml 1N Folin ciocalteu reagent and 10 ml saturated Na_2CO_3 solution was added and volume made up to 100 ml with water. The content of flask was mixed well and the color was measured after 30 min at 760 nm against experimental blank adjusted to 0 absorbancy. A standard graph was plotted using absorbance and concentration of tannic acid. For the sample, 5g was

ePrint@CFTRI

boiled for 30 min with 400 ml of water, cooled, transferred to a 500 ml volumetric flask and diluted to mark. The sample was mixed well and filtered through Watman filter paper. The clear filtrate was used for estimation after suitable dilution following the same procedure as for the standard. The total phenolics as tannic acid equivalent were calculated from the standard graph.

Phytates

Phytic acid was estimated according to the HPLC method of Tangendjaja et al. (1980). Phytic acid was extracted from samples (1g) with 3% trichloroacetic acid (25 ml) for 30 min in a mechanical shaker and the slurry was centrifuged for 20 min at 18,000 rpm. The supernatant liquid was filtered through a 0.22 µm Millipore filter, and an aliquot (25µl) was injected into a µ Bondapak C_{18} column; the eluent solvent was sodium acetate (0.005 M) at a flow-rate of 2 ml/min. Pure phytic acid (from corn) was used as standard. The HPLC was carried out using a Water Associate HPLC System consisting of Binary gradient pumping system for solvent delivery and Photodiode Array Detector Model 2996 (UV, 254nm,) with a Millennium data processor.

Trypsin Inhibitor Activity

Trypsin inhibitor activity was determined according to the method of Kakade et al. (1974) as follows.

Reagents:

Tris buffer: Tris (hydroxymethyl aminomethane) 1.21 and 0.59 g of $CaCl_2$ were dissolved in 180 ml of distilled water. The pH was

ePrint@CFTRI

adjusted to 8.2 with 1 N HCl and made up to 200 ml with distilled water. This solution was pre-warmed to 37°C for BAPNA formulation.

BAPNA solution: BAPNA (Nα-Benzoyl-DL-Arginine-ρ-Nitroanilide) (0.8 g) was dissolved in 2 ml of dimethylsulfoxide and diluted to 200 ml with Tris buffer. BAPNA solution is stable up to 4h.

Trypsin solution: 10 mg trypsin was dissolved in 250 ml 0.001 N HCl and stored in cold (5-10°C). Preferably fresh solution should be prepared with each run.

Acetic acid (30%): 30 ml of concentrated acetic acid was made up to 100 ml with distilled water.

Preparation of sample: One gram of finely ground (100 mesh) defatted sample was extracted with 50 ml of 0.01 N NaOH for 3h at room temperature and then centrifuged. The supernatant was used for estimation after suitable dilution.

Procedure: Aliquots ranging from 0.2 to 1.0 ml were pipetted into duplicate test tubes and volume was adjusted to 2.5 ml with distilled water. To each tube 1.5 ml of trypsin solution was added, placed in a water bath at 37°C and 5 ml of previously warmed (37°C) BAPNA solution was added. Exactly after 10 min the reaction was terminated by adding 1.0 ml of acetic acid (30%). The absorbance was measured at 410 nm against the reagent blank. The reagent blank was prepared by adding 1.0 ml of acetic acid to test tubes containing trypsin and water (2.5 ml) followed by addition of 5.0 ml of BAPNA solution. The rest of the procedure was the same. Sample blanks also prepared using sample extract. Trypsin inhibitory unit per ml (TIU/ml) vs.

ePrint@CFTRI

volume of extract was plotted and extrapolated to zero. One trypsin unit was defined as an increase in 0.01 absorbance units at 410 nm per 10 ml of reaction mixture under condition used. The following formula was used to calculate TIU/g of sample:

$$\text{TIU/g Sample} = \frac{\text{Y intercept (TIU/ml)} \times \text{dilution factor}}{\text{Sample weight (g)}}$$

Trypsin inhibitor activity was expressed in terms of trypsin units inhibited (TIU) and the value expressed as TIU/ mg of sample.

Nitrogen Solubility

The nitrogen solubility was determined according to the method of Mattil (1971). Samples (1g) was mixed with 15 ml of water and the pH of slurry adjusted to desired pH by the addition of 2N HCl or 2N NaOH. The volume was adjusted to 20 ml with distilled water. It was shaken for 1h at room temperature (27°C), centrifuged at 5000 rpm for 20 min and the pH of the supernatant noted. Aliquots of 5.0 ml of the extract were used for nitrogen estimation by the Kjeldahl method. The solubilized nitrogen was expressed as percent of the total nitrogen of the meal.

Non-Protein Nitrogen (NPN)

NPN was estimated using 10% TCA to precipitate the protein as the procedure given by AOAC (1984). One gram of flour was dispersed in 10 ml of 10% trichloroacetic acid and shaken for one hour using rotary shaker and centrifuged at 5000 rpm for 20 min. The clear supernatant was used for nitrogen estimation by micro-kjeldahl method and calculated as percent of non-protein nitrogen in the sample.

ePrint@CFTRI

Available Lysine

Available lysine was determined by the FDNB-reactive lysine method of Carpenter (1960). One gram of finely ground sample (containing about 12 mg reactive lysine) was taken into a round-bottom flask and four anti-bumps glass beads and 10 ml of 8% sodium bicarbonate solution was added and shaked gently by hand until sample is fully wet. 15 ml of FDNB solution (0.4 ml FDNB dissolved in 15 ml ethanol) was added, stoppered and shaken on a mechanical shaker for 2h. Ethanol was removed later by evaporating on a boiling water bath, cooled and then 30 ml of 8.1N HCl added and refluxed for 16h.

The hydrolysate was filtered while still hot and made up to 250 ml. A 2.0 ml of clear filtrate was pipetted into each of two stoppered test tubes A and B. The contents of tube B were extracted with 5 ml diethyl ether. The ether layer removed and discarded as much as possible using a dropping pipette. The tube placed in hot water (about 80°C) until effervescence from residual ether has ceased and then cooled. One drop phenolphthalein solution was added and then the NaOH solution from a dropping pipette until the first pink appears. Two ml of pH 8.5 carbonate buffer (19.5 g $NaHCO_3$ and 1g Na_2CO_3 in 250 ml water and adjust pH to 8.5) and 5 drops of methoxycarbonyl chloride was added. The tube was stoppered, shaken and left for about 8 min. Concentrated HCl (0.75 ml) was added and the remaining gas was removed by gentle shaking. The content of tube was extracted three times with diethyl ether as described above and the residual ether was removed by placing the tube in hot water, cooled and volume made up to 10 ml with water. During the pauses between the manipulations of tube B, tube A was

ePrint@CFTRI

extracted three times with diethyl ether and the residual ether was removed as before. The tube was cooled and the volume made up to 10 ml with 1N HCl. The absorbance of the tubes A and B was measured against water at 435 nm. Reading A minus reading B (blank) is the net absorbance attributable to DNP-lysine.

Two ml working standard DNP-Lysine solution (314 mg of mono Є-N-dinitrophenyl-lysine hydrochloride monohydrate was dissolved in 250 ml of 8N HCl and then 10 ml was diluted to 100 ml with water which containing 0.1 mg of lysine in a 2 ml aliquot) was taken into the tubes A and B and then experimental procedure was repeated with all above steps. The available lysine was calculated as follows:

$$\text{FDNB reactive lysine (g/16g N)} = \frac{W_s \times A_t \times v \times 100 \times 100 \times C_f}{W_t \times A_s \times a \times P}$$

Where, "W_s" is weight of standard as mg lysine in 2 ml; "W_t" is weight of test material in mg; "A_s" is net absorbance of standard; "A_t" is net absorbance of sample, "v" is volume of filtrate hydrolysate; "a" is aliquot of filtrate taken for analysis; "P" is % protein (N×6.25) in test sample; "C_f" is correction factor for hydrolysis losses (1.09 can be used for materials virtually free of carbohydrates and 1.2 for vegetable materials).

In vitro Protein Digestibility

The *in vitro* protein digestibility was determined by the method described by Akeson and Stahman (1964). Pepsin followed by pancreatin digest was prepared by incubating 100 mg of protein equivalent sample with 1.5 mg pepsin in 15 ml of 0.1N HCl at 37°C

ePrint@CFTRI

for 3 h. After neutralization with 0.2N NaOH, 4 mg Pancreatin in 7.5 ml of Phosphate buffer of pH 8.0 was added. One ml of toluene was added to prevent microbial growth and the solution was incubated for additional 24h at 37°C. An enzyme blank was prepared in the same way without the sample. After 24h, the enzyme was inactivated by the addition of 10 ml 10% TCA to precipitate undigested protein.

The volume was made up to 100 ml and centrifuged at 5000 rpm for 20 min. The protein content of the clear supernatant was determined by Kejldhal method. The *in vitro* digestibility was calculated as the percentage of the total protein solubilised after enzyme hydrolysis. The following equation was used for calculation:

$$\text{\% Protein Digestibility} = \frac{\text{(Protein in supernatant – Protein in enzyme blank)}}{\text{(Protein in sample – TCA soluble protein at zero h)}} \times 100$$

Degree of Hydrolysis

The degree of hydrolysis in protein hydrolysates was determined according to the method of Adler-Nissen (1979). 0.25 ml of a sample containing between 0.25×10^{-3} and 2.5×10^{-3} amino equiv/L, was mixed in a test tube with 2 ml of phosphate buffer at pH 8.2 (0.2125 M NaH_2PO_4 was added to 0.2125 M Na_2HPO_4 until pH is 8.20 ± 0.02; The proportion of volumes was approximately 43:1000). Two milliliter of 0.1% trinitrobenzenesulfonic acid (TNBS) solution (TNBS was dissolved in deionized water in a volumetric flask covered with aluminum foil; the solution must be prepared immediately before use) was added and the test tube was shaken and placed in a water bath at 50 ± 1°C for 60 min. During incubation the test tubes and the water bath was covered with aluminum foil

ePrint@CFTRI

because the blank reaction is accelerated by exposure to light. After 60 min, 4 ml of 0.1 N HCl was added to terminate the reaction and the test tube was allowed to stand at room temperature (cooling below room temperature may cause turbidity because of the SDS) for 30 min before absorbance was read against water at 340 nm. The reaction on the blank and the standard solutions were carried out by replacing the sample with 1% SDS and 1.5×10^{-3} M L-leucine in 1% SDS respectively. The absorbance of the blank and the standard were determined same as above.

Amino Acid Analysis

Amino acid analysis was performed according to the method of Bidlingmeyer et al. (1984) using a Water Associate Pico-Tag amino acid analysis system.

The amino acid analysis was carried out using a three-step procedure. In the first step the protein samples were acid hydrolyzed to obtain free amino acid residues, amino acids were then modified by PITC (Phenylisothiocyanate) in the second step and last step includes the separation of the modified amino acids by RP-HPLC.

Hydrolysis of Protein: An aliquot of 50 µl of reverse phase purified samples were pipetted in to a tube (6×50 mm Pyrex ™) and placed in the special vacuum vial. The vacuum vial was then attached to the Waters Associates Pico-Tag workstation manifold and the samples were dried under 50-60 mtorr vacuum. After drying, the vacuum was released and 200 µl of constant boiling HCl (6N) containing phenol 1% (v/v) was pipetted in to bottom of the vacuum vial. The vacuum vial was then reattached to the manifold, evacuated and sealed under vacuum. Samples were hydrolyzed in the workstation at 110°C for 24 h.

ePrint@CFTRI

After hydrolysis the residual HCl inside the vacuum vial was removed under vacuum. Standard free amino acids as a mixture (Pierce H) containing up to 25 nmol of each amino acid were placed in the tubes (6×50 mm) and dried under vacuum. Free amino acids and hydrolyzed samples were dried down under vacuum after adding re-drying solution (10-20 µl) containing ethanol: water: triethylamine (TEA) (2:2:1) to each tube. When the vacuum reached 50-60 mtorr, the samples were ready for derivatization.

Derivatization of hydrolyzed amino acids: The derivatization reagent was made fresh each time and consisted of ethanol: triethylamine: water: PITC (7:1:1:1). The PITC was stored at -20°C under nitrogen to prevent the formation of breakdown products. To make 300 µl reagents, sufficient for 12 samples, 210 µl of ethanol was mixed thoroughly with 30 µl aliquots of PITC, TEA and water. PTC amino acids were formed by adding 20 µl of the reagent to the dried samples and sealing them in the vacuum vials for 20 min at 25 ± 2°C. The excess of reagents were then removed under vacuum using the workstation. When the vacuum reached 50-60 mtorr, the samples were ready for analysis by RP-HPLC.

Chromatography: The HPLC was carried out using a Water Associate HPLC system consisting of a binary gradient pumping system and photodiode array detector (Model 2996) with a Millennium data processor. The temperature was controlled at 38 ± 1°C with a column heater. Samples were injected in volumes ranging from 5-50 µl. The column was an application specific Pico-Tag column (150×3.9 mm).

ePrint@CFTRI

Solvent system: Solvent system consisted of two eluents, (A) an aqueous buffer 0.14 M Sodium acetate containing 0.5 ml/l of TEA, titrated to pH 6.4 with glacial acetic acid: acetonitrile (94:6) and (B) 60% acetonitrile in water. A gradient of 0-46% B in 10 min was used for separation. After the run a washing step in 100% B was included so that the residual sample components were removed. The PTC amino acids were detected at 254 nm. The gradient elution programme is shown in Table 13 and the separation of a standard amino acid mixture is shown in the Figure 14.

Estimation of Tryptophan

Protein was extracted according to the method of Concon (1975) and estimated the tryptophan by the method described by Swakais and Pest (1990). One gram of defatted sample was taken in a 25 ml propylene test tube with caps and 10 ml of 0.075 N NaOH was added, mixed until no lumps. The dispersion was shaken for 30 min and centrifuged at 5000 rpm for 10 min and the supernatant was transferred into a clean test tube. To 0.5 ml of supernatant, 5 ml of ninhydrin reagent (1.0 g of ninhydrin in the mixture of concentrated HCl of 37% plus formic acid of 96% at a ratio of 2:3) was added and incubated at 35°C for 2h. After incubation, the solution was cooled to room temperature and volume made up to 10 ml with ethyl ether, vortexed, filtered and a clear filtrate was read at 380 nm. A standard tryptophan curve was prepared using 0-100µg tryptophan. From the standard graph, the concentration of tryptophan was calculated and expressed as g/100g protein.

ePrint@CFTRI

Table 13. The Gradient Program for Amino Acid Analysis

Time (min)	Flow (ml)	%A	%B	Curve
0.01	1.0	100	0	
10.00	1.0	54	46	7
11.00	1.0	0	100	6
13.00	1.0	0	100	6
14.00	1.0	100	0	6
25.00	1.0	100	0	6

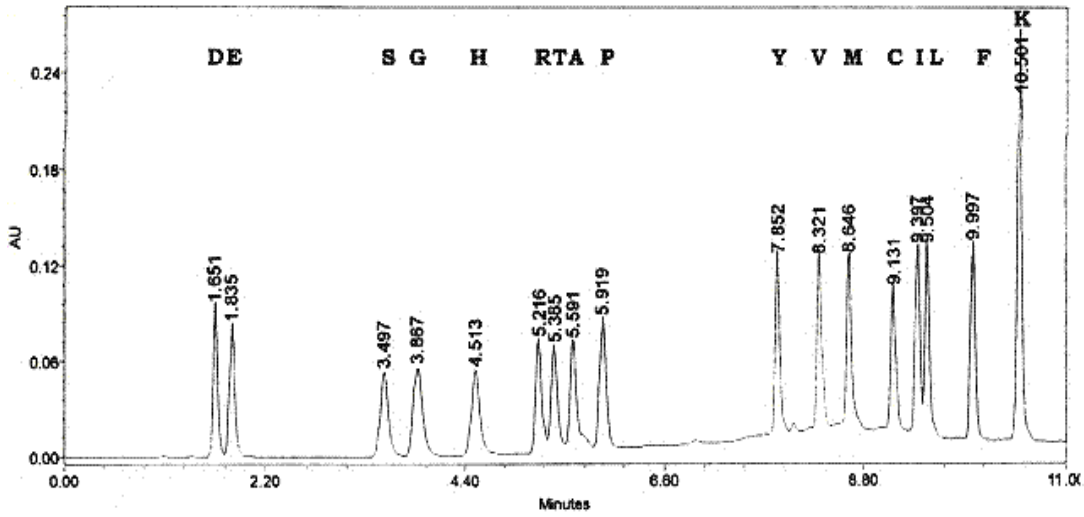


Fig 14. Elution Profile of PTC-Amino Acids Using a Pico-Tag Amino Acid Analysis System. The amino acids are represented by the single letter code. Column: Pico-Tag™ (150×3.9 mm, 4 µl); flow rate: 1.0 ml/min; wave length: 254 nm; temperature: 38°C; injection volume: 5 µl (312.5 pmol of each amino acid); solvent A: 140mM Na acetate, 3.6 mM triethylamine (TEA), pH 6.4/ acetonitrile (96/4); solvent B: 60% acetonitrile.

ePrint@CFTRI

Chemical Score

Chemical score which is based on the amount of the most limiting amino acid present in the test protein relative to the amount of the amino acid in reference protein (egg) was calculated using following formula (FAO, 1968).

$$\text{Chemical Score} = \frac{\text{g EAA in test protein} \times \text{g total EAA in egg}}{\text{g total EAA in test protein} \times \text{g EAA in egg}} \times 100$$

Essential amino Acid Index

EAA index was calculated according to the procedure of Oser (1951) taking into account the ratio of EAA in the test protein relative to their respective amounts in whole egg protein using following formula.

$$\text{EAA Index} = \sqrt[10]{\frac{100a \times 100b \times \cdots\cdots \times 100j}{a_e \times b_e \times \cdots\cdots \times j_e}}$$

Or,

$\log \text{EAA index} = 1/10 \left(\log 100a / a_e + \log 100b / b_e + \cdots\cdots + \log 100j / j_e\right)$

Where,

a, b, ..., j = % of each EAA in food protein (N×6.25)

a_e, b_e, ... , j_e = % of respective EAA in whole egg protein

Biological Value (BV)

The BV was calculated according to the method of Oser (1959) using the following formula:

$$BV = 1.09 \text{ (EAA index)} - 11.7$$

ePrint@CFTRI

Nutritional Index (NI)

Nutritional index (NI) was calculated by using the formula given by Crisan and Sands (1978).

$$NI = \frac{\text{EAA Index} \times \% \text{ Protein content}}{100}$$

Computed Protein Efficiency Ratio (C-PER)

The C-PER was calculated from the digestibility and amino acid composition data according to the procedure of Satterlee et al. (1979). The different steps involved are as follows:

1. The *in vitro* digestibility of samples and reference casein was determined according to the procedure of Akeson and Stahman (1964).
2. The essential amino acid composition of the sample was used from the amino acid composition data and the essential amino acid composition of casein from the FAO/WHO, 1973 (Table 14).
3. The essential amino acid (EAA) percentage was calculated as percentage of the FAO/WHO (1973) standard, using the following equation.

$$EAA\% = \frac{\text{EAA content}}{\text{FAO/WHO standard}} \times \text{Protein digestibility}$$

4. Weights were assigned to each EAA% as shown in Table 15 and computed using the following formula:

$$X = \sum 1/\ EAA\% \times \text{associated weight}$$

ePrint@CFTRI

$$Y = \sum \text{Weight}$$

5. The sum of weight (Y) was divided by the sum of the weighted reciprocals (X) for the sample and for the casein reference to obtain the "EAA score" for each.
6. The "EAA score" of the sample was divided by the "EAA score" of the casein reference to express the sample protein as a ratio of casein standard (SPC).
7. The C-PER was calculated using following formula:

$$\text{C-PER} = -2.1074 + 7.1312\ (\text{SPC}) - 2.5188\ (\text{SPC})^2$$

Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

This was calculated according to the method of Sarwar and McDonough (1990) using the essential amino acid composition of the test sample and the amino acid pattern suggested by FAO/WHO (1991) for different age groups. The essential amino acid composition of the sample was analyzed by HPLC. *In vitro* protein digestibility was determined by the method of Akeson and Stahman (1964).The uncorrected amino acid score for the nine essential amino acids were determined by dividing the mg of an essential amino acid in 1 g of test protein to mg of same amino acid in 1 g of reference protein (FAO/WHO pattern). Finally, multiplying the least of the uncorrected amino acid scores by the digestibility value to give the PDCAAS score. PDCAAS values above 100 were considered as 100

ePrint@CFTRI

Table 14. FAO/WHO Standard (1973) for each EAA

EAA	g EAA/100 g protein
Lysine	5.5
Methionine + Cysteine	3.5
Threonine	4.0
Isoleucine	4.0
Leucine	7.0
Valine	5.0
Phenylalanine + Tyrosine	6.0
Tryptophan	1.0

Table 15. Weight Assigned for Different Amount of EAA%

EAA%	Weight	EAA%	Weight
100%	1.00	41 - 50%	11.31
91 - 99%	2.00	31 - 40%	16.00
81 - 90%	2.83	21 - 30%	22.63
71 - 80%	4.00	11 - 20%	32.00
61 - 71%	5.66	0 - 10%	45.25
51 - 60%	8.00		

ePrint@CFTRI

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE at alkaline pH (8.3) was carried out according to the method of Laemelli (1970) in a discontinuous buffer system. Vertical slab gel electrophoresis was carried out in a BROVIGA mini model electrophoresis unit, at 25 ± 2°C.

Reagents:

A. Acrylamide (29.2 g) and bis-acrylamide (0.8 g) were dissolved separately in minimum amount of water and mixed and made up to 100 ml, filtered and stored in a dark brown bottle at 4°C.

B. Separating gel buffer (1.5 M, pH 8.8): Tris (18.15 g) was dissolved in water and pH adjusted to 8.8 with 6N HCl, solution made up to 100 ml and stored at 4°C.

C. Stacking gel buffer (0.5 M, pH 6.8): Tris (3 g) was dissolved in water and pH adjusted to 6.8 with 6 N HCl, solution made up to 100 ml and stored at 4°C.

D. Sodium dodecyl sulphate (10%): SDS (10 g) was dissolved in water (100 ml).

E. Ammonium persulphate (10%) was freshly prepared by dissolving 50 mg in 0.5 ml of distilled water.

F. Tank buffer: Tris (0.3 g), Glycine (1.44 g) and SDS (0.1 g) were dissolved in 100 ml of distilled water.

G. Staining solution: Coomassie brilliant blue (CBB) R-250 (0.1 g) was dissolved in a mixture of methanol: acetic acid: water (25:10:65, v/v). The reagent was filtered and stored at 25 ± 2 °C.

H. Destaining solution: Methanol: acetic acid: water (25:10:65, v/v).

ePrint@CFTRI

I. Sample buffer: Prepared in solution C diluted 1:4, containing SDS (4% w/v), β-mercaptoethanol (10% v/v), glycerol (20% v/v) and bromophenol blue (0.1% w/v).

Initially contents of the separating gel (Table 16) were mixed and TEMED was added, degassed and poured between the assembled glass plates, the bottom edge sealed with agar (1 % w/v). The gels were layered with 0.5 ml of distilled water and allowed to polymerize at 25 ± 2 °C for 30 min. After polymerization of the separating gel, contents of stacking gel (Table 16) were mixed and layered above the polymerized separating gel. The gels thus prepared were of size 10.5 × 9 cm and thickness 0.8 mm.

Table 16. Preparation of Separating Gel and Stacking Gel

Solution	Separating gel (ml)			Stacking gel (ml)
	7.5% T 2.7%C	10% T 2.7%C	12% T 2.7% C	5% T 2.7% C
Solution A	2.5	3.33	4.00	0.85
Solution B	2.50	2.50	2.50	0.00
Solution C	0.00	0.00	0.00	1.25
Distilled water	4.85	2.02	3.35	3.00
Solution D	0.10	0.10	0.10	0.05
TEMED	0.01	0.01	0.01	0.01
Solution E	0.05	0.05	0.05	0.03

ePrint@CFTRI

Samples were prepared by dissolving protein (10 mg) in solution "I" (1ml). The samples were heated in a boiling water bath for 5 min. cooled samples were then layered in the wells immersed in solution "F" (Tank buffer) and were run at constant voltage (50 V) until the tracking dye, bromophenol blue entered the lower tank buffer. Low range protein M_r markers (Ovalbumin, 43 kDa; Carbonic Anhydrase, 29 kDa; Soybean Kunitz Inhibitor, 20 kDa; Lysozyme, 14.3 kDa; and Aportinin, 6.5 kDa) were used. The markers were supplied as a solution having a total protein concentration of 3 mg/ml. The markers were diluted 1:1 with the solution "I" and boiled in a boiling water bath for 5 min.

Staining: The gels were stained for protein with reagent "G" for 3-4 h at 25 ± 2°C and destained in the reagent "H".

Scanning Electron Microscopy

Scanning electron microscopy studies of different samples were carried out using LEO 435 VP, Cambridge model surface scanning electron microscope. Before loading the samples into the system, the samples were coated with gold using Poloron SEM coating system E-5000. Average coating time was 2-3 min. Thickness of coating was 200-300 nm, which was calculated using following formula:

$$T = 7.5\ It$$

Where,

I = Current in mA, t = time in min and T = Thickness in A°. The coated samples were loaded on the system and the image viewed under 20 kV potential using 35 mm Picoh camera.

ePrint@CFTRI

Color Measurement

The color measurement was done for samples using Minolta CM 3500D (Japan) instrument using visible wavelength. Colors of samples were measured by C-illuminating 2D view angle. The value of *L* (lightness), *a* (redness and greenness), *b (*blueness and yellowness) was measured using Hunter Color System (Fig 15).

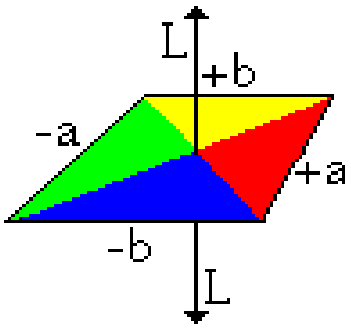


Fig 15.Hunter Color System

Dietary Fibre

Dietary fibre was determined according to the method of Nils et al. (1983) as follows:

Reagent: (1) 0.1M sodium phosphate buffer, pH 6.0; (2) 4M HCl; (3)4 M NaOH; (4) 95% ethanol; (5) 78% ethanol; (6) acetone

Enzymes: (1) Termamyl 60L; (2) pepsin; (3) panceratin

Procedure:

(1) one gram of sample was weighted and transferred to an Erlenmeyer flask and 25ml of 0.1M sodium phosphate buffer (pH 6.0) was added and the sample suspended thoroughly.

ePrint@CFTRI

(2) Termamyl (100 µl) was added to the flask, covered and incubated in a boiling water bath for 15 min with occasional shaking.

(3) The flask was cooled and 20ml distilled water was added, pH adjusted to 1.5 with HCl and 100 mg of pepsin was added. The flasks were covered and incubated at 40°C in a water bath with agitation for 60 min.

(4) After incubation, 20ml of distilled water was added and pH adjusted to 6.8 with NaOH and 100 mg of panceratin was added. The flasks were covered and incubated at 40°C in a water bath with agitation for 60 min.

(5) The pH was adjusted to 4.5 with HCl and sample filtered through a dry and weighted crucible (porosity 2) containing 0.5g Celite (exact weight known) as the filter aid. The crucible contents were washed with 2×20 ml of distilled water.

(A) Residue (Insoluble fibre)

The residue was washed with 2×10 ml of 95% ethanol and 2×10 ml acetone and dried at 105°C to constant weight. After cooling the crucibles were weighted (D_1) and incinerated at 550°C for at least 5h, cooled and weighted again (I_1).

(B) Filtrate (soluble fibre)

The volume of combined filtrate and water washings were adjusted to 100 ml and 400 ml warm (60°C) 95% ethanol was added. The solution was allowed to precipitate for 1h and filtered through a dry and weighted crucible containing 0.5 g of Celite. The residues were

ePrint@CFTRI

washed with 2×10 ml of 78% ethanol, 2×10 ml of 95% ethanol, and 2×10 ml of acetone. The crucibles were dried at 105°C overnight. Crucibles were cooled and weighted (D_2) and incinerated at 550°C for at least 5h. After cooling the crucibles were weighted again (I_2).

Blank: Insoluble and soluble blank values were obtained by following the same procedure without sample (B_1 and B_2).

The insoluble and soluble fibre was calculated using following formula:

$$\% \text{ Insoluble dietary fibre} = \frac{D_1 - I_1 - B_1}{W}$$

$$\% \text{ Soluble dietary fiber} = \frac{D_2 - I_2 - B_2}{W}$$

Where,

W = sample weight (g), D= Weight after drying (g),

I = weight after incineration (g), B = weight of ash free blank (g)

Water Absorption Capacity (WAC)

Water absorption capacity was determined according to the method of Sosulski (1962). 1 g sample was taken in a centrifuge tube which had been weighted previously. 6 ml of distilled water was added and stirred with a glass rod. The glass rod was washed further with 2 ml water, taking care to see that no protein adhered to the centrifuge tube. After 30min, the suspension was centrifuged at 3000 rpm for 25min; the supernatant liquid was discarded and kept inclined at an angle of 45° in an oven at 50°C for 20min, then

ePrint@CFTRI

transferred to a dessicator and weighed. The differences in the two weights gave the amount of water absorbed by the material. Water absorption capacity is expressed as the amount of water absorbed by 100 g of the material.

Fat Absorption Capacity (FAC)

The fat absorption capacity of samples was measured by the method described by Sosulski et al. (1976). Six ml refined sunflower oil (Gemini, Maharashtra, India) added to 1g of sample in a calibrated, graduated tube. The contents were mixed for 5min. After 30min the contents were centrifuged at 3000 rpm for 25 min and the volume of free oil was measured. Fat absorption capacity is expressed as the amount of oil (ml) bound by 100 g of sample.

Foam Capacity (FC) and Foam Stability (FS)

FC and FS were determined by the method of Lawhon and Carter (1971). Protein sample (3 g) was taken in 100 ml distilled water and the slurry was adjusted to pH 7. This was quantitatively transferred in to a blender and whipped for 5 min at high speed. The slurry was poured immediately into 250 ml measuring cylinder and the total volume and the liquid volume were measured after 30 sec. The difference in the volume is the foam and is expressed as percent foam capacity. The foam stability determined by measuring the volume of foam after 30 min and is expressed as foam stability.

Emulsion Capacity (EC)

Emulsion capacities of samples were determined by the procedure of Beuchat et al. (1975). To 1.5 g of sample, 25 ml of glass double distilled water was added and blended at low speed (10,000 rpm) for 30 sec at 27°C using the bajaj standard blender. At

ePrint@CFTRI

this point, refined sunflower oil (Gemini, Maharashtra, India) was added from a burette at a constant rate of 5.0 ml/min with continuous blending. The addition of oil continued until the breakpoint, indicated by separation of the oil from aqueous phase was reached. The total amount of oil delivered from the burette was noted. The EC was expressed as ml of oil emulsified by 1.0g of sample.

Bulk Density

Bulk densities of samples were measured by the method of Jones and Tung (1983). A calibrated glass centrifuge tube was accurately weighed. It was filled with sample to 5 ml and weighed again. From the difference in weights, the bulk density of samples was calculated and expressed as g/ml.

Spaghetti Cooking Quality

To evaluate the cooking quality of spaghetti samples, 10 g of raw spaghetti was cooked in 250 ml of boiling distilled water for optimum cooking time (10 min). The following parameters were measured to evaluate the cooking quality of the different spaghetti samples:

(a) Cooked weight

Cooked weight was the weight of 10 g of dry spaghetti after cooking (Manthey and Hareland, 2001)

(b) Cooking loss (total solids in gruel)

Cooking loss of different spaghetti samples was carried out according to ISI method (IS 1485, 1993) with some modifications. Ten g of spaghetti was broken into length of ~5 cm and was cooked in 200 ml of boiling distilled water. Spaghetti was cooked to its optimal cooking time with occasional stirring. After cooking the

ePrint@CFTRI

sample was rinsed with stream of distilled water (around 50 ml) for about 30 sec in a Buchner funnel and allowed to drain for 2 min. Total volume of gruel and the rinsed water collected, was measured. The gruel was stirred well for even distribution of the solid content. 20 ml of gruel was pipetted out into a tarred Petri dish and evaporated to dryness on a water bath. The Petri dish was transferred to a hot air oven maintained at 105 ± 2°C and dried to constant mass. The cooking loss was calculated using following formula:

$$\text{Total solid in gruel, percent by mass} = \frac{(M_2 - M_1) \times V}{2}$$

Where,

M_2 = mass in g of Petri dish with total solid

M_1 = mass in g of empty Petri dish, and

V = volume of gruel in ml

(c) Spaghetti firmness

A Universal Texture Measuring System (Twin screw material testing, M/C, UTM LLOYDS, LR-5K, U.K) was used for measurement of spaghetti firmness according to the method of Walsh and Gilles (1971). Cooked spaghetti samples were immediately transferred into 250 ml beaker containing distilled water at room temperature. Two cooked spaghetti strands were removed from the water and compressed within 0.5 mm distance from base plate at a 90° angle using a specially designed aluminum probe (Fig 16). The force required to shear the spaghetti was measured in triplicate and average values were calculated. A higher shear value indicates a firmer product.

ePrint@CFTRI

Fig 16. Universal Texture Measuring System

ePrint@CFTRI

(d) Spaghetti Stickiness

Surface stickiness of the cooked spaghetti was determined according to the method Dexter et al. (1983) as standardized by Aalami and Leelavathi (2006). A Universal Texture Measuring System (Twin screw material testing, M/C, UTM LLOYDS, LR-5K, U.K) with a special plunger and sample holder (Fig 17**)** was used for the measurement of spaghetti stickiness.

Ten gram of uncooked spaghetti were broken into 5 cm long strands and cooked for 10 min in 150 ml of distilled water. Cooked spaghetti was drained for 1 min over a Buchner funnel and loaded on a polished aluminum plate side-by-side to cover an area about 5 cm wide.

Stickiness test was started 13 min after cooking. The plunger was fastened to the instrument cell, which was attached to a 5 kg load transducer. The condition used throughout the testing included a crosshead speed of 4 mm/min and a compression force of 8 Newton (~816g). The force generated during the compression of cooked spaghetti, and on lifting the plunger, was graphically plotted on the computer screen (Fig 18). Stickiness was defined as the maximum depression (negative value) recorded during lifting of the plunger. The negative value was calculated into N/M^2 using a factor of 10500 for a compression force of 8 Newton (~ 816g), calculated based on contact surface of the plunger.

ePrint@CFTRI

Fig 17. Stickiness Measurement of Spaghetti Samples by the Universal Texture Measuring System

ePrint@CFTRI

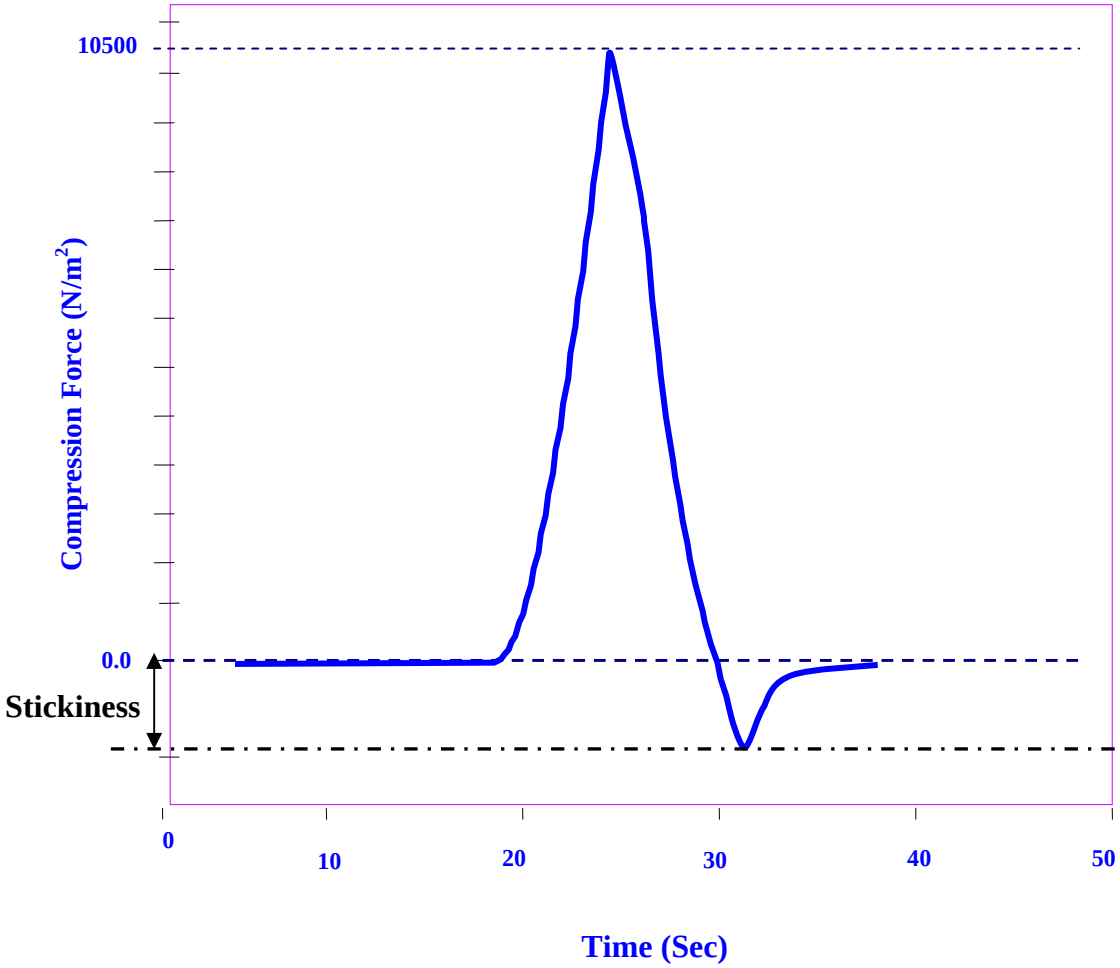


Fig 18. Stickiness Graph

ePrint@CFTRI

Farinograph Characteristics of Semolina

The farinograph characteristics of semolina were determined using Brabender farinograph-E (Brabender, OHG, Germany) according to the method of Irvine et al. (1961). The modified software version 2.3.2 which is specific for the measurement of farinograph characteristics of semolina was supplied by manufacturer and used in this study. Fifty grams of the sample (on 14% moisture basis) was placed in the farinograph mixer bowl and the temperature was maintained at 30°C. Sample was mixed for 1 min, then the required amount of water (35%) was added and test run for 20 min. The farinograph characteristics of semolina and enriched semolina samples were obtained from farinograms of respective samples.

Sensory Evaluation

Sensory evaluation was carried out on the cooked spaghetti by a panel of 10 trained judges. The panelist was asked to score different quality characteristics including color and appearance (0-10 points), taste and flavor (0-10 points), texture (0-20 points), mouthfeel (0-10 points) and overall quality (50 points). Analysis of variance was used to test the difference between the parameters.

Statistical Analysis

The collected data were subjected to analysis of variance (ANOVA) by Duncan's Multiple Range Test (DMRT) to compare any significant difference among the means values (Snedecor and Cochran, 1967).

ePrint@CFTRI

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 defatted meal contains about 35-40% protein with a well balanced amino acid and excellent functional properties. However, the use of mustard/rapeseed protein as a source of protein is limited by the presence of undesirable toxic and anti-nutritional compounds such as glucosinolates, phytates and phenolics compounds. The hulls present in the meal contribute high fiber content.

The mustard/rapeseed meal is potentially a high quality protein supplement but its use in the diet of monogasteric animals is limited by the relatively high content of indigestible fiber in the meal (Slominski et al., 1994). The low digestibility of rapeseed is partly due to hull fraction that is high in insoluble dietary fiber such as lignins (Bjergegaard et al., 1991).

In most of the plant seeds, fiber and tannins are concentrated in the seed hull (Dietrych and Oleszek, 1999). Therefore dehulling of seeds improves the nutritional quality of the meal, decreases crude fiber level and improves the nutritive value of the meal (Sosulski and Zadernowski, 1981).

ePrint@CFTRI

With this above background, (1) a process developed by CFTRI to dehull the mustard/rapeseed has been used to prepare dehulled mustard meal; (2) fractionation was used to reduce the hull fraction in commercial mustard.

1) Dehulling of Mustard Seed

Mustard variety T-59 is grown on a large scale in India. Based on the consistent performance and high yields it was released for general cultivation in the year 1968-69 throughout the country at different locations. The variety was locally named as Varuna (T-59). This variety is suitable for different environmental conditions (Singh et al., 1976). Therefore it was of interest to collect comprehensive data on the dehulling aspect of this variety. In addition, the chemical, toxic and anti-nutritional constituents present were evaluated. A trial with 10 kg batch of this variety was carried out by the method outlined in Figure 9 (section II) to determine the yield of dehulled kernel, hull, other fractions and oil content (Fig 19 to 22). The yield data of dehulling process is given in Table 17.

Dehulling resulted in three fractions consisting of 72% dehulled kernel, 18% hulls and 10% fine and germ. The ratio of hulls plus fine and dehulled seed was 28:72. Jones (1979) reported a ratio of 27:71 for hulls plus fines and dehulled seed after dehulling and air classification of Bronowski cultivar of rapeseed. Sosulski and Zadernowski (1981) have reported a value of 18-23, 76-79 and 30-36% for hulls, meals and flours, respectively for rapeseed dehulled by front-end dehulling.

ePrint@CFTRI

Fig 19. Mustard Whole Seed

Fig 20. Dehulled Kernel

ePrint@CFTRI

Fig 21. Mustard Hull

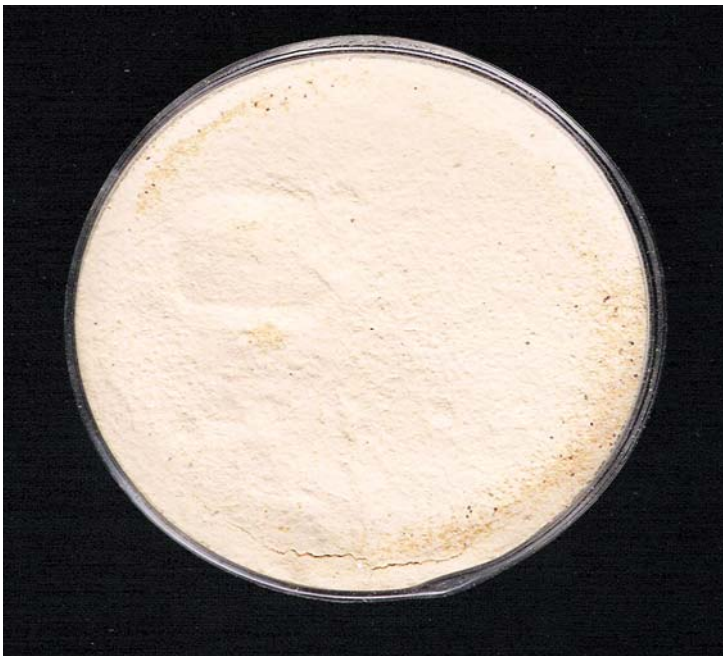


Fig 22. Dehulled Defatted Meal

ePrint@CFTRI

Table 17. Material Balance of Dehulled Mustard Seeds (10 kg Batch)

Constituents	Amount (kg)	Percent (%)
Graded Seed	10	100
Hulls	1.8 ± 0.05	18 ± 0.5
Dehulled Seed + Fines	8.2 ± 0.1	82 ± 1
Fine + Germ	1.0 ± 0.02	10 ± 0.2
Dehulled Kernel	7.2 ± 0.1	72 ± 1
Oil	3.7 ± 0.5	37 ± 0.5
Dehulled Defatted Meal	3.5 ± 0.1	35 ± 1

Mean ± SD of two different trials

ePrint@CFTRI

The results are comparable to those reported values. However, the variation may be due to the size of the seeds and varietal differences.

The dehulled kernels contained 37% oil was defatted with n-hexane by repeated extraction. The yield of defatted meal was 35% by weight (Table 17).

Liu et al. (1995) showed that the small seed have a higher proportion of hulls than the big seeds and thus are higher in fiber and lower in fat content. Triple low varieties with yellow hulls have received much attention due to their lower fiber and more digestible non-starch polysaccharides (NSP) and lignin content (Downey and Bell, 1990; Slominski and Campbell, 1990; Liu et al., 1995). The largest proportion of NSP and lignin are present in hull (Liu et al., 1995). Bell (1993) reported that yellow-seed *B. campestris* had a lower percentage of seed coat (hull) than brown seed type. Yellow-husked strains of rapeseed contain less hull and the hull contains less crude fiber and lignin than in brown hulls strains (Bell, 1984).

Mechanical dehulling of rapeseed/mustard seed for removal of hull have been reported by other investigators (Ekuland et al., 1971; Durkee, 1971; Jones, 1979; Schneider, 1979; Jones and Holme, 1982; Mieth et al., 1983a; Mieth et al., 1983b; McCurdy, 1990; Bell, 1993; Mawson et al., 1993; Liu et al., 1995; Kracht et al., 1999; Zeb et al., 2002).

The chemical composition of mustard meal as compared to whole seed is given in Table 18.

ePrint@CFTRI

Table 18. Chemical Composition of Mustard Whole Seed (T-59 Variety) and Dehulled Defatted Meal

Constituents (%)	Whole Mustard Seed	Dehulled Defatted Meal
Moisture	5.6 ± 0.4	8.0 ± 0.3
Fat	37.7 ± 0.3	1.0 ± 0.2
Protein (N×6.25)	22.0 ± 0.5	48.0 ± 0.5
Ash	4.2 ± 0.1	5.2 ± 0.2
Crude Fiber	10.0 ± 0.5	3.0 ± 0.3
Carbohydrate (by diff.)	19.5 ± 0.5	35.8 ± 0.5

Mean ± Standard Deviation (SD) of three determinations

ePrint@CFTRI

The protein and oil content of mustard seed was 22 and 38%, respectively. The values are comparable with reported values by Anand et al. (1976) for this variety. Dehulling and defatting the mustard seed increased the protein content to 48% and decreased the fat content to 1%. Dehulling decreased the crude fiber content from 10 to 3% indicating that the fiber content being reduced by 70%.

Depending on the variety and environmental conditions, the chemical composition of rapeseed/mustard seed is different. Maheshwari et al. (1981) reported that the oil and protein content of 44.7, 42.7, 37.2% and 23.8, 23.8 and 27.5% for *B. napus*, *B. campestris* and *B. juncea*, respectively. Mackenzie (1973) reported that the oil composition of five cultivars of *B. juncea* namely Bragonde, Ekla, L22A, Primus and Stroke were 33.8, 31.8, 34.9, 37.1 and 40.9%, respectively.

El-Nockrashy et al. (1977) have reported the protein content of 24.3, 24.1% and 43.1, 39.4% for the whole seed and defatted meal of Erglu and Lesira varieties of *B. napus,* respectively. The oil and protein content are comparable to the reported values for different varieties (El-Nockrashy et al., 1977; Maheshwari et al., 1981).

Kracht et al. (2004) reported that dehulling of the rapeseed decreased the crude fiber content in the meal and cake by approximately 40% and increased the protein content by 7% and 13%, respectively. Sosulski and Zadernowski (1981) studied on the fractionation of rapeseed meal into flour and hull components and the influence of hull percentage on meal composition. The result indicated that there was a progressive reduction in crude fiber and

ePrint@CFTRI

increase in protein content, with decrease in hull percentage among the three *Brassica* species.

The protein contents of hulls are highly variable, perhaps because of the difficulty to separate completely hulls from embryos (Bell and Shires, 1982). The protein content of the hulls was in the range of 12-16% depending on the efficiency of separation (Finlayson, 1976).

Table 19 shows the anti-nutritional factors present in mustard whole seed and dehulled defatted meal. The mustard whole seed contained lower amounts of anti-nutritional constituents which increased after dehulling and defatting of the mustard kernel. The results clearly indicate that these anti-nutritional factors concentrate in the meal after dehulling and defatting. The whole mustard seed contained 10.2 and 7.85 mg/g of isothiocyanate and oxazolidinethione, respectively. After dehulling and defatting they were increased to 18.75 and 13.75 mg/g in meal. These constituents are concentrated in dehulled defatted meal due to the removal of hull and oil.

Glucosinolates are widely distributed among the different parts of *Brassica* plant and the maximum concentrations are found in the seed embryo at full maturity. The factors that affect glucosinolate content are the genetical, cultural and environmental factors associated with its growth, age of the plant and variety (Fenwick et al., 1983). The rapeseed species are not identical with respect to their glucosinolate content and different varieties contain different amount of glucosinolates.

ePrint@CFTRI

Table 19. The Content of Anti-Nutritional Factors Present in Mustard Whole Seed and Dehulled Defatted Meal

Constituents	Whole seed (T-59 variety)	Dehulled Defatted Meal
Isothiocyanates (mg/g)	10.20 ± 0.20	18.75 ± 0.30
5-Vinyloxazolidine-2-thione (mg/g)	7.85 ± 0.15	13.75 ± 0.35
Phytic Acid (%)	3.55 ± 0.05	5.60 ± 0.10
Phenolics (%)	1.20 ± 0.05	2.12 ± 0.06
Trypsin Inhibitor Activity (TIU/mg Protein)	6.8 ± 0.05	3.8 ± 0.1

Mean ± SD of three determinations

ePrint@CFTRI

Jones (1979) reported the isothiocyanate and oxazolidinethione content of three *B. napus* variety as 4.08 and 12.77 mg/g for Target, 0.087 and 0.29 mg/g for Tower and 0.48 and 0.66 mg/g for Bronowski, respectively. The isothiocyanate and oxazolidinethione content of 3.2 and 13.5 mg/g was reported for Lesira variety of *B. napus* (El-Nokrashy et al., 1977).

Robbelen and Thies (1980) have reported that dehulling increased the glucosinolate content of the meal. The seed coats or hulls contain relatively small amount of glucosinolates (Josefsson, 1970) and this explain the increase in glucosinolates after dehulling of seed. Mawson et al. (1993) have reported that the dehulling process are generally ineffective in reducing rapeseed glucosinolates and dehulling increases the relative proportion of glucosinolates in the remaining meal.

The oxazolidinethione content of T-59 variety is comparable to the reported values. However, the isothiocyanate content is much higher than reported values for different varieties (El-Nockrashy et al., 1977; Jones, 1979; Mayton, 1996).

The phenolics and phytic acid content of whole mustard seed was 1.2% and 3.55%, respectively which increased to 2.12 and 5.6% after dehulling and defatting (Table 19).

Esterified phenolics acids are the predominant phenolics acid present in rapeseed protein products as they constitute up to 80% of the total phenolic acids. Trans-sinapic acid is the predominant phenolic acid found in rapeseed/canola.

ePrint@CFTRI

Sinapic acid constituted 71-97% of the phenolic acids liberated from the soluble fraction of the esterified phenolic acids in rapeseed/canola meal. Sinapine, the choline ester of sinapic acid is the most abundant phenolic choline ester present and occurs in rapeseed meals in the range of 1.0-2.5% (Mueller et al., 1978). Liu et al. (1995) reported that most of sinapine in rapeseed is concentrated in the cotyledons and hulls contain only small amount of it. Dehulling result in significant increases in the concentration of sinapine in the meal (Fenwick et al., 1984). On the other hand most of the condensed tannins are concentrated in the seed coat (hull) and dehulling reduces the tannin content in the meal (Mawson et al., 1993). Tannins in rapeseed are mostly located in seed coat (Mansour et al., 1993).

Phytic acid typically exists in rapeseed as salts of Ca, Mg and K. It is found in the crystalline globoids (0.5-2.8 μm in size) inside protein bodies in the cells of the radicle and primarily the cotyledon. The phytin globoids may account up to 10% weight of the seed. Therefore it can be expected that dehulling and defatting increases the content of phytates in the resulting meal (Mills and Chong, 1977; Yiu et al., 1982; Yiu et al., 1983). The phytic acid content of 2-4% for the whole rapeseed and 2-5% for defatted meal has been reported. In general, the phytic acid content of the whole mustard seed and defatted meal are in good agreement with the values reported by others for different varieties (Shah et al., 1976; Gillberg and Tornell, 1976a; Gillberg and Tornell, 1976b; Nwokolo and Bragg, 1977; Jones, 1979; Reddy et al., 1982; Tzeng et al., 1988a; Tzeng et al., 1988b; Tzeng et al., 1990).

ePrint@CFTRI

There are reports about trypsin inhibitor activity in the rapeseed meal. The trypsin inhibitor activity has been shown to be associated with low molecular weight protein of mustard/rapeseed (Gururaj Rao et al., 1978; Mieth et al., 1983a; Prakash and Narasinga Rao, 1986; Mandal et al., 2002).

The trypsin inhibitor activity in the whole seed was 6.8 TIU/mg of protein and it reduced to 3.8 TIU/mg of protein in dehulled defatted meal. The decrease in trypsin inhibitor activity in the meal may be due to removal of hulls during dehulling which reduces the tannin content in the meal.

Fernandez et al. (1982) have reported that heat stable tannins show trypsin inhibitor activity. Gururaj Rao et al. (1978) have reported that low molecular weight protein from mustard (*B. juncea*) variety Varuna had a trypsin inhibitor activity of 9.8 TIU/mg proteins.

2) Fractionation of Commercial Cake

The removal of hull produces meal with lower fiber, high protein contents and reduces subsequent processing costs. But removal of the hull of rapeseed/mustard is expensive due to the small size of the seeds. Hence dehulling presently has limited economic viability (Gunston, 2004). Mechanical methods for separation of hulls from rapeseed/mustard seeds are inefficient and dehulling is not a standard practice in rapeseed/mustard oil extraction plants (Naczk et al., 1994). Currently, rapeseed/mustard is crushed in Europe, Asia, north and South America. However, the conventional oilseed milling industries are not designed for the separation of hulls.

ePrint@CFTRI

In India most of the seeds produced are used for oil extraction by Ghanies and expellers. The seeds are often processed in the villages without a pre-heat treatment in small plants and the oil is used for cooking and the press cake is used as animal feed or fertilizer. The processing conditions are favorable for the hydrolysis of glucosinolates to toxic hydrolysis products.

To overcome the problem of high hull (high crude fiber) content in commercial mustard cake a suitable method was used as outlined in Figure 9. This method includes incorporation of a conditioning step before crushing of seed to activate the enzyme myrosinase. The glucosinolates are hydrolyzed when the wet, unheated plant material is crushed (VanEtten et al., 1969). The enzyme presents in the seeds come into close contact with the glucosinolates when the seeds are crushed during extraction of the oil.

The temperature (40-70°C) and moisture content (13% and above) are favorable for enzymatic hydrolysis of glucosinolates before oil extraction (Maheshwari et al., 1981). This process yields oil with high pungency and the cake with lower amounts of glucosinolates.

However, the cake obtained still contain high amount of crude fiber. In order to overcome this problem, the defatted cake was separated using a Quadromat Mill® into three different fractions (Fig 23 to 26). The material balance data for this process is presented in Table 20. A 10 kg batch process yields around 3.55 kg high pungency oil, 7.6 kg cake with 13% oil , 2.1 kg low hull fraction with fine particles (fine fraction), 3.3 kg low hull fraction with larger particles

ePrint@CFTRI

(coarse fraction) and 1.0 kg high hull fraction. The cake obtained was dark brown in color. The low hull fine and coarse fractions were light yellow in color compared to cake.

VanMegen (1983) reported that most of the hulls which are responsible for high fiber content of the meal, could be removed by a simple sieving step giving an almost white powder. Tape et al. (1970) proposed pin milling and air classification of rapeseed cake after desolventization, into protein rich and fiber rich fractions. The principal drawback of this process were: (a) the need for double or triple milling to obtain a reasonable yield of protein rich fraction and, (b) the limited enrichment which is obtained in the fine fraction.

ePrint@CFTRI

Fig 23. Commercial Mustard Cake

Fig 24. Powdered Commercial Cake

ePrint@CFTRI

Fig 25. Low Hull Fraction of Cake

Fig 26. High Hull Fraction of Cake

ePrint@CFTRI

Table 20. Material Balance Data of Fractioned Commercial Mustard Cake

Constituents	Amount (kg)	Percent (%)
Commercial Seed	10	100
Oil (Ghani Press)	2.35 ± 0.1	23.5 ± 1
Cake	7.6 ± 0.15	76 ± 1.5
Oil (Solvent Extracted)	1.2 ± 0.08	12 ± 0.8
Defatted Cake	6.4 ± 0.1	64 ± 1
Low Hull High Protein (Fine Fraction)	2.1 ± 0.06	21 ± 0.6
Low Hull High Protein (Coarse Fraction)	3.3 ± 0.05	33 ± 0.5
High Hull Low Protein Fraction	1 ± 0.05	10 ± 0.5

Mean ± SD of two different trials

ePrint@CFTRI

A process for separating the slurry of finely ground defatted meal and hexane by liquid cyclone processing into hulls (cyclone unders) and flour (cyclone overflow) has been patented by Sosulski and Zadernowski (1980). This process claimed to provide a practically hull free flour.

The chemical composition of whole seed, cake and its fractions are given in Table 21. 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 21). These two fractions were combined and named as **"low hull high protein fraction"**. 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-nutritient content of seed, cake, low hull high protein and high hull fractions are presented in Table 22.

ePrint@CFTRI

Table 21. Chemical Composition of Mustard Seed, Cake and its Fractions

Constituents	Whole seed (Commercial Variety)	Cake	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

Mean ± SD of three determinations

ePrint@CFTRI

Table 22. Anti-Nutritional Factors Present in Whole Seed, Cake and Its Fractions

Constituents	Whole Seed (Commercial Variety)	Cake	Low Hull Fraction of cake	High Hull Fraction of cake
Isothiocyanates (mg/g)	8.1 ± 0.05	2.14 ± 0.06	1.69 ± 0.07	1.14 ± 0.06
5-inyloxazolidine-2-thione (mg/g)	5.9 ± 0.05	4.95 ± 0.05	2.08 ± 0.04	1.36 ± 0.05
Phenolics (%)	1 ± 0.1	1.3 ± 0.1	1.8 ± 0.05	1.0 ± 0.05
Phytates (%)	3.1 ± 0.05	3.8 ± 0.1	5.3 ± 0.08	4.4 ± 0.05
Trypsin Inhibitor Activity (TIA/mg Protein)	2.2 ± 0.03	3.95 ± 0.05	3.5 ± 0.1	4.5 ± 0.05

Mean ± SD of three determinations

ePrint@CFTRI

It was observed that fractionation and defatting of commercial cake increased all the anti-nutritional constituents in the cake fractions. The results were similar to that of dehulled mustard meal. However, the amount of isothiocyanate and oxazolidinethione in whole seed were 8.1 and 5.9 mg/g which reduced to 1.69 and 2.08 mg/g in the low hull fraction, respectively (Table 22). This may be due to the extraction of these hydrolyzed products into the oil. Studies by Daun and Hougen (1977) and Ohlson (1976) have shown that if the hydrolysis of glucosinolates occurs during crushing before the oil extraction, the sulfur-containing hydrolytic products, isothiocyanates and oxazolidine- thione, enter the oil. Ballester et al. (1970) have reported a mean value of isothiocyanate and oxazolidinethione of 1.4 and 7.6 mg/g respectively for 12 samples of presscake meal of rapeseed (*Brassica napus*) collected from local factories.

The enzyme activation during crushing causes the hydrolysis of glucosinolates and its hydrolyzed products can be extracted along with oil resulting in high pungency oil. Glucosinolates are highly soluble in water, but not in organic solvent. However the hydrolyzed products are less soluble in water but readily soluble in organic solvents (Kozlowska et al., 1972; Mieth et al., 1983a). Thus lower amounts of isothiocyanate and oxazolidinethione in cake may be expected compared to the whole seed. In addition, removal of oil by solvent extraction further reduced these anti-nutritional constituents.

Defatting increased the content of phenolics, phytates and trypsin inhibitor activity in the cake compared to the whole mustard seed. The amount of phytates and phenolics in whole seed were 3.1 and 1% and increased to 3.8 and 1.3% in cake, respectively. The phytates and phenolics content of low hull fraction of cake were 5.3

ePrint@CFTRI

and 1.8%, respectively (Table 22). These results clearly indicated that defatting and removal of hull increased the concentrations of these anti-nutritional factors because these constituents are mostly located in seed cotyledon.

The trypsin inhibitor activity in cake was 3.95 TIU/mg of protein and it reduced to 3.5 TIU/mg of protein in low hull fraction after defatting and oil extraction. The reduced trypsin inhibitor activity of this fraction compared to the cake is may be due to low content of hull in this fraction. The hull contained higher amounts of tannin which have been shown to inhibit the trypsin activity (Fernandez et al., 1982).

The high hull fraction of cake contained 1.14 and 1.36 mg/g of isothiocyanate and oxazolidinethione, respectively. While the phytate and phenolic contents of this fraction were 4.4 and 1.0%, respectively. In general, the concentrations of these anti-nutritional constituents were lower than the low hull fraction of cake. However, the trypsin inhibitor activity was higher (4.5 TIU/mg protein) compared to low hull fraction (3.5 TIU/mg protein).

The lower content of toxic and anti-nutritional factors in high hull fraction may be due to high content of hull in this fraction and also lower concentration of these constituents in the hull. Higher trypsin inhibitor activity of this fraction may be due to high content of tannin in the hull. These results correlate well with the high hull content in this fraction.

ePrint@CFTRI

Mustard/rapeseed meal has been considered as a potential source of food-grade proteins with a well-balanced amino acid composition (Ohlson and Anjo, 1979). However the presence of glucosinolates, phytates, phenolics and hull limited the use of rapeseed meal as a protein source in food/feed purposes.

A number of methods have been reported to isolate protein from mustard/rapeseed like alkaline extraction followed by precipitation, extraction of the protein in different solvents and isoelectric precipitation, alkaline extraction and neutralization, counter-current extraction and isoelectric precipitation, ultrafiltration, diafiltration and ion exchange purification and protein micellar mass (PMM) procedure (Gillberg and Tornell, 1976a; Gillberg and Tornell, 1976b; Kantharaj Urs and Kowsalya, 1976; Lonnerdal et al., 1977; El-Nokrashy et al., 1977; Tzeng et al., 1988a; Tzeng et al., 1988b; Ismond and Welsh,1992).

None of these methods were satisfactory in removing all the toxic and anti-nutritional constituents completely. Also some of these methods are not suitable for commercial production because of lower yields. Therefore, it was necessary to develop a unique method involving multiple steps to remove all the anti-nutritional constituents during isolation and to obtain protein with higher purity and yield.

Recently we have reported and patented a method for preparation of protein isolate with reduced toxic and anti-nutritional factors (Alireza Sadeghi et al., 2004; Alireza Sadeghi et al., 2006).

The parameters followed and standardized are: protein solubility as a function of pH, extraction of protein at alkaline pH, meal to solvent ratio, NaCl concentration, time of extraction,

ePrint@CFTRI

activated carbon treatment, protein recovery by heat coagulation and washing.

Standardization of Parameters for Isolation and Detoxification of Protein from Mustard Meal/Cake

For the study, T-59 variety of mustard (*Brassica juncea*) was used. The seeds were dehulled and defatted to obtain the meal (Fig 6, Section II). The Ghani pressed commercial cake was defatted and fractionated to obtain low hull high protein fraction (Fig 9, Section II). The meal and low hull fraction were used to isolate the protein.

1. Protein Solubility

The protein solubility profile of dehulled defatted mustard meal as a function of pH in water is presented in Figure 27. The solubility profile of mustard meal showed two minima at pH 3.8 and 7.8 and the solubility increases with increasing the pH. The maximum solubility of >80% was observed above pH 11. It is very clear from the solubility graph (Fig 27) that precipitation of protein at solubility minima is not a satisfactory method for the recovery of proteins since almost 40% still remains in solution.

Gururaj Rao et al. (1978) have studied the extractability of protein from mustard flour in various aqueous solvents, namely water, 1M NaCl and 2% sodium hexamethaphosphate (SHMP) solution.

ePrint@CFTRI

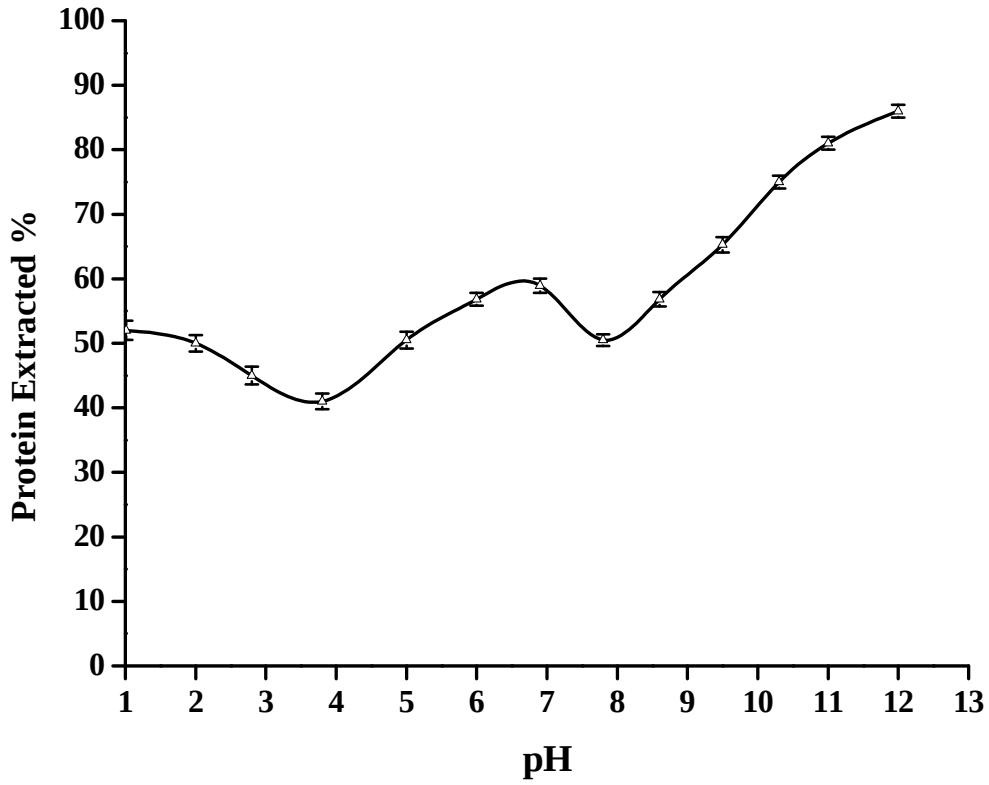


Fig 27. Protein Solubility Profile of Dehulled Defatted Meal at Different pH Values

ePrint@CFTRI

The solubility profile of mustard proteins in water and in 1M NaCl solution showed two minima at pH 3.8 to 4.0 and 7.8 to 8.0. Finnigan and Lewis (1985) have reported that rapeseed protein showed two solubility minima one at pH 4.0 and another at pH 8.0. It has been reported that extraction of proteins from defatted rapeseed meal at alkaline condition and isoelectric precipitation resulted in low yield of isolate. The low yield was attributed to the wide range of molecular weight and isoelectric points in mustard proteins (Lonnerdal and Janson, 1972; Gillberg and Tornell, 1976a).

Unlike other protein-rich oilseed such as soybean, peanut and sunflower seed, rapeseed has a complex protein composition and contains proteins with widely different isoelectric points and molecular weights (Lonnerdal et al., 1977). Therefore, the production of isoelectrically precipitated rapeseed protein isolate with 90% protein content requires complex processes which results in low yields. Generally the products contain high amount of phytates, present as protein-phytate complexes. Thus, traditional protein isolation processes are not feasible and technically unattractive for the production of high-quality mustard/rapeseed proteins. Diosady et al. (1989) have reported that sodium hydroxide solutions are effective solvents for mustard/ rapeseed proteins extraction and give high extraction yields but the isoelectric precipitation of these extract results in lower yields and low protein content in isolate.

2. Extraction of Protein at Alkaline pH

Figure 28 shows the solubility profile of protein and phytic acid at alkaline pH range (10.0-12.5). The extraction of meal protein increased with increase in pH. At pH 11, around 81% of meal protein was extracted and the extractability of phytic acid was negligible.

ePrint@CFTRI

Therefore in this investigation, the extraction of protein at pH 11 was used for further investigation. The results are in close agreement with that reported by others (Gillberg and Tornell, 1976a; Gillberg and Tornell, 1976b; Tzeng et al., 1990).

Tzeng et al. (1988a) have reported that the aqueous NaoH solution at pH 11 increased nitrogen extractability up to 85%. The strong alkaline condition extracts high amount of nitrogen and decrease the extractability of the phytic acid and leave most of it in the residual meal (Diosady et al., 1987). However, extraction of protein at very high alkaline pH results in amino acid destruction and reaction between non-protein compounds and rapeseed protein. Higher pH values (>12) and high temperature are favorable for the formation of lysinoalanine. In addition, glucosinolate and their breakdown products have a strong affinity to protein, especially at higher pH (Bjorkman, 1973; Deng et al., 1990; Rubin et al., 1990). It has been suggested that tannins may not bind proteins at high pH because the phenolic groups of tannins are ionized and are unavailable for hydrogen bonding (Loomis and Battaile, 1966). Phytic acid was also reported to have low extractability at high pH from soybean meal (DeRham and Jost, 1979). The low extractability is mainly due to a shift in the equilibria of ternary complexes due to the presence of excess sodium ion (Cheryan, 1980).

ePrint@CFTRI

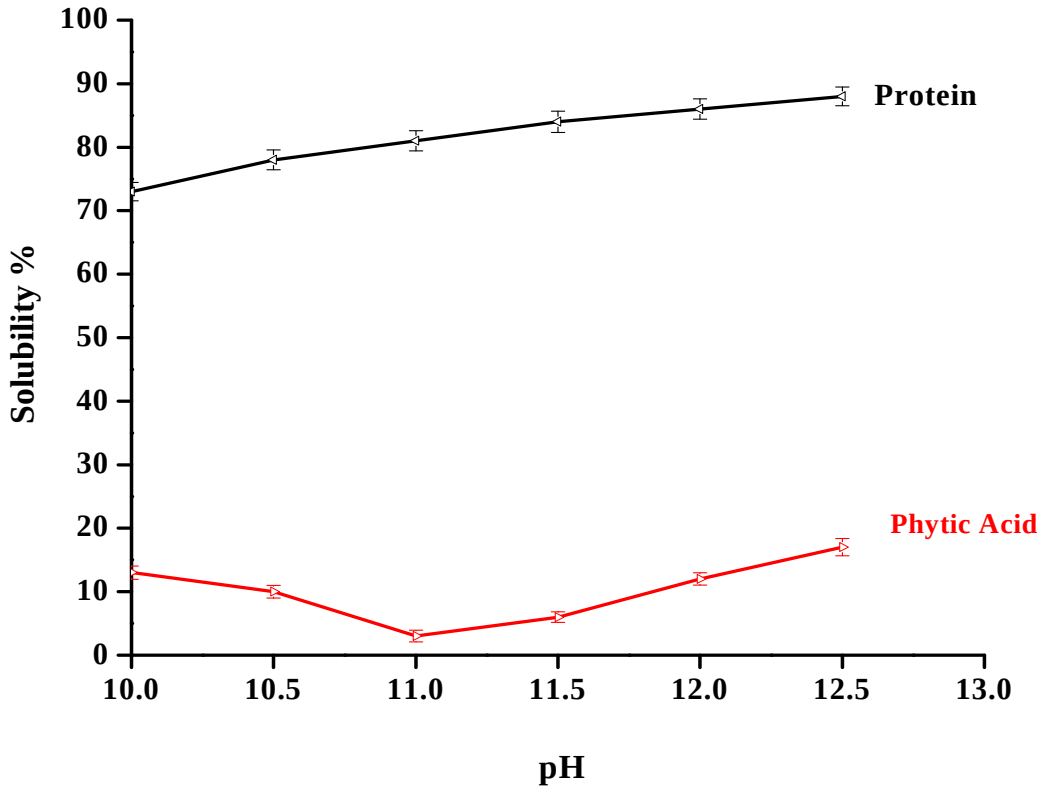


Fig 28. Solubility of Protein and Phytic Acid at Different Alkaline pH Values

ePrint@CFTRI

3. Meal and Solvent Ratio

The second important factor in the extraction of protein is the ratio of meal to solvent to assess the possibility of transforming the process to a large scale where the volume of effluent has to be minimized. To study the effect of this parameter on extractability of protein, pH 11 and shaking time 60 min was used. The ratio of meal to solvent 1:5, 1:10, 1:15, 1:20 and 1:25 (w/v) was used. Table 23 shows the results of extraction of meal protein at pH 11 with different ratio of meal to solvent. The results indicated that the extractability of mustard protein at pH 11 increased with increasing the ratio of meal to solvent and reached a maximum extractability of 89% at 1:25 ratio of meal to solvent. However, extracting the protein at a ratio of 1:25 may not be feasible for industrial scale. Therefore, a ratio of 1:15 (w/v) has been selected for extraction of protein.

4. NaCl Concentration

The solubility behaviors of each protein are known to be affected by pH, salt concentration and ionic strength (Kinsella, 1976). The most common salt used in solubility studies are sodium chloride and calcium chloride.

Table 24 shows the result of protein extraction with different NaCl concentration ranging from 0.1 to 0.5 M, at pH 11, meal to solvent ratio of 1:15 and shaking time of 30 min. The results indicated, as the concentration of salt increased the extractability of nitrogen was also increased from 81 to 90%. However there was not any significant difference ($P< 0.05$) between extraction of protein using 0.4 and 0.5 M NaCl concentration.

ePrint@CFTRI

Table 23. Effect of Meal to Solvent Ratio on the Extractability of Protein at pH 11

Meal: Solvent Ratio (w/v)	Protein Extracted (as % of Total Protein)
1:5	52.6 [e]
1:10	68.5 [d]
1:15	81 [c]
1:20	85 [b]
1:25	89 [a]

* Values followed by different letters are significantly different (P<0.05)

ePrint@CFTRI

Table 24. Effect of NaCl Concentration on the Extraction of Protein

NaCl Concentration (M)	Protein Extracted (%)
0.0	81 ± 0.5 [e]
0.1	85.3 ± 0.7 [d]
0.2	86.4 ± 0.8 [c]
0.3	88 ± 0.5 [b]
0.4	89.5 ± 0.3 [a]
0.5	90.2 ± 0.3 [a]

* Values followed by different letters are significantly different (P<0.05) for three determinations

** Extraction pH 11; Ratio of meal to solvent 1:15 (w/v)

*** Mean ± SD of three determinations

ePrint@CFTRI

Another important factor is to be studied was the effect of salt concentration on the amount of protein loss in supernatant after recovery of protein by heat coagulation (Table 25). The results showed that the loss of protein after heat coagulation using 0.1 M NaCl was minimum and it was increased with the increase in salt concentration. From the above results it can be concluded that 85% of protein could be extracted at pH 11 and 0.1 M NaCl concentration with minimum loss of protein (8%) in whey.

Prakash and Narsinga Rao (1986) have reported that the presence of various electrolytes had a profound effect on extractability of mustard/rapeseed protein. Klockeman et al. (1997) reported that protein solubility of canola waste in water was <40% at pH 3.0-11.0, which increased to 50 and 70% when NaCl was added to the solutions at a concentration of 0.1 and 1.0 M. Gururaj Rao et al. (1978) reported that the solubility of mustard protein at all pH values above pH 4 in 1M NaCl solution was higher than in water. The solubility in 0.1M NaCl showed intermediate values between water and 1M NaCl.

Sosulski and Bakal (1969) reported that the protein in rape, turnip rape, flax and sunflower meals were progressively less water-soluble than soybean but were correspondingly more soluble in salt solutions. Increased nitrogen solubility at alkaline pH and different ionic environment has been reported by Quinn and Jones (1976).

Numbers of reports are available in the literature for reduction of the phytic acid content in the rapeseed protein by extraction with the different concentration of sodium chloride and ultrafiltration technique (Siy and Talbot, 1982; Kroll et al., 1991).

ePrint@CFTRI

Table 25. Effect of NaCl Concentration on the Protein Loss after Recovery of Protein by Heat Coagulation

NaCl Concentration (M)	Protein Loss (%)
0.1	8 ± 0.2 [e]
0.2	9.2 ± 0.1 [d]
0.3	10.1 ± 0.2 [c]
0.4	11.3 ± 0.1 [b]
0.5	12.7 ± 0.2 [a]

* Values followed by different letters are significantly different (P<0.05) for three determinations

** Mean ± SD of three determinations

ePrint@CFTRI

Diosdy et al. (2003) have reported that the salt (NaCl) increases the ionic strength of extraction solution thereby breaks apart the ionically bonded phenolic-protein complexes and increase the quantity of free phenolic compound present in the spent extraction solution. Addition of sodium chloride was reported to reduce the amount of sinapic acid bond to canola protein at pH 4.5 (Rubino et al., 1996).

5. Time of Extraction

The solubility profile of mustard proteins at different pH showed two isoelectric points with an optimum pH 11 for extraction of protein. Addition of NaCl at 0.1M concentration also resulted in higher extraction and was efficient in inhibiting the phenolics and phytates reaction with proteins. Use of high alkaline condition has been shown some negative effect on the quality of protein, leads to formation of lysinoalanine and racemization of amino acids, and favors the reaction between protein and anti-nutritional constituents like isothiocyanate (Paquet and Ma, 1989; Deng et al., 1990; Bjorkman, 1973).

Therefore, effect of extraction time on the extractability of protein was studied. The mustard meal proteins were extracted at optimum pH 11 using 0.1M NaCl solution and meal to solvent ratio 1:15 w/v. The time of extraction was varied from 15-60 min. the results are presented in Table 26. As it can be seen from the table, 85.3% of protein was extracted at 30 min and increasing the extraction time up to 60 min did not show any significant improvement in the protein extraction.

ePrint@CFTRI

Table 26. Effect of Extraction Time on Protein Solubility

Shaking Time (min)	Protein Extracted (%)
15	82 ± 0.5 [b]
30	85.3 ± 0.7 [a]
45	86.2 ± 0.3 [a]
60	87 ± 0.2 [a]

* Values followed by different letters are significantly different (P<0.05) for three determinations

** Mean ± SD of three determinations

ePrint@CFTRI

Xu and Diosady (1994) reported that a maximum extraction of rapeseed protein at 30 min under alkaline condition and above which the extraction did not improve the protein solubility.

6. Activated carbon treatment

Activated carbon has been used for many years to eliminate organoleptic defects in grape juice wine. Use of activated carbon appears to provide a more controllable, effective and perhaps cost effective approach to eliminate odor, off flavor and color. Charcoal in its present form has been used historically in a wide range of both food and medical products and presently is being used in water treatment and purification systems to impair sensory quality and food safety.

Activated carbon has an extraordinarily large surface area and pore volume that gives a unique adsorption capacity (Baker et al., 1992). Activated carbon has both chemical and physical effects on substances where it is used as a treatment agent as (i) adsorption; (ii) mechanical filtration; (iii) ion exchange and (iv) surface oxidation.

There have been some reports about the use of activated carbon in food processing. Activated carbon was used to remove off color and polyphenols from white grape juice concentrate. Polyphenols such as reservatrol and pyenogerols are powerful antioxidants and are concentrated in grapes (Sovak, 2001). Activated carbon is a strong absorbent of phenols (Anderson, 1946) and is efficient in removing the tannins from grape juice. Activated carbon can remove colors due to the tannins.

ePrint@CFTRI

Activated carbon is a good absorber of cations and used medicinally to absorb a wide variety of toxins. Activated charcoal along with bentonite has been shown to reduce the amount of the mycotoxin aflatoxin in milk and patulin in apple juice (Sands et al., 1976; Doyle et al., 1982).

The presence of anti-nutritional constituents in mustard/ rapeseed protein limits its use in feed or food. Therefore, in the present study activated carbon treatment was selected for removal of these undesirable constituents. Hence, the effect of different levels of activated carbon, contact time and pH of treatment on the removal of anti-nutritional constituents was studied. After the extraction of protein at pH 11 with 0.1M NaCl solution, the protein solution was treated with different levels of activated carbon ranging from 0.5 to 3% (w/v), with different contact time from 30 min to 2h. The pH of protein solution was maintained at acidic, neutral and alkaline. The results showed a maximum removal of more than 98% of all the anti-nutritional constituents by treating with 2% (w/v) activated carbon at the pH 7 and contact time of 1h.

Based on the optimized conditions in the present investigation protein isolate was prepared from the mustard meal by extracting at pH 11, using 0.1 M NaCl solutions with meal to solvent ratio 1:15 and extraction time of 30 min. The pH was re-adjusted to 7 and solution was treated with 2% (w/v) activated carbon with a contact time of 1h.

Woyewoda et al. (1978) demonstrated that activated carbon was effective in removing glucosinolate hydrolysis products and colored compounds without protein loss. They have reported that

ePrint@CFTRI

carbon treatment alone in the pH range from 3 to 10 removed over 93% (Maximum 98% of pH 3) of isothiocyanates. The carbon treatment was also partially effective in nitrile removal but glucosinolates were not eliminated.

Activated carbon treatment has also been successfully used to remove phenolic compounds and phytates in the preparation of soybean protein and peanut protein (Brooks and Morr, 1982; Seo and Morr, 1985). How and Morr (1982) studied the effect of activated carbon on removal of phenolic compounds from soy protein products and showed that activated carbon effectively removed phenolic acids from dilute alkali solution with an optimum contact time 30 min. Tzeng et al. (1988b) developed a method which includes extraction of rapeseed proteins using aqueous sodium hexametaphosphate solution followed by activated carbon treatment, ultrafiltration, diafiltration and purification by ion-exchange. The protein isolate obtained had about 90% protein (N×6.25), free of glucosinolates low in phytate, light in color and bland to taste.

7. Recovery of Protein

The traditional methods of protein recovery by alkaline extraction and isoelectric precipitation are not economically and technically feasible for rapeseed/mustard because the meal has a complex protein composition with different isoelectric points. Therefore, the production of isoelectric precipitated protein isolate with 90% protein requires complex processes (Lonnerdal et al., 1977; Diosady et al., 1989).

In the present investigation, the solubilized protein was recovered by different methods; viz. (1) isoelectric precipitation (2)

ePrint@CFTRI

alcoholic precipitation (3) ultrafiltration and (4) heat coagulation (by steam injection). The effect of these methods on yield and removal of anti-nutritional constituents were evaluated.

Table 27 shows the data on recovery of protein by different methods. In all the cases the protein after recovery was washed and spray dried except the ultrafiltration where the protein solution after concentration (concentration factor 10) was spray dried without washing.

The yield of protein by isoelectric and alcoholic precipitation was 45 and 41%, respectively and was low compared to other methods. Recovery of protein by heat coagulation and ultrafiltration gave higher yields of 60 and 65%, respectively. However, in the ultrafiltration method, the removal of phytates and phenolics was 90 and 78%, respectively and was much lower than other methods with lower protein content of 56%. The higher removal of phenolics and phytates in other methods may be due to washing of protein before spray drying.

The spray dried protein obtained after ultrafiltration showed slightly darker color compared to other methods.

Among the methods used to recover proteins, heat coagulation was suitable in terms of purity of protein, yield and removal of anti-nutritional factors. Heat coagulation almost completely eliminated the isothiocyanate and oxazolidinethione. In addition, 99% of phytates and phenolics were removed in the protein. The purity of the protein was higher (95%) compared to other methods (Table 27).

ePrint@CFTRI

Table 27. Isolation and Recovery of Protein by Different Methods and Their Effects on Yield and Removal of Anti-nutritional Factors

Parameter		Ultrafiltration	Alcohol Precipitation	Isoelectric Precipitation	Heat Coagulation
Protein (%)		56.2 ± 0.8	83.7 ± 0.5	88 ± 0.5	95 ± 0.5
Yield of Protein (as % of Total Protein)		65	41	45	60
Removal of Anti-Nutritional Factors	Isothiocyanates	98 ± 0.3	99 ± 0.2	98 ± 0.5	99.5 ± 0.1
	5-Vinyl-Oxazolidine-2-thione	98.8 ± 0.2	99.6 ± 0.1	98.8 ± 0.4	100%
	Phytates (%)	90 ± 0.5	97 ± 0.3	95 ± 0.5	99 ± 0.3
	Phenolics (%)	78.2 ± 0.5	96 ± 0.2	96 ± 0.3	98.7 ± 0.3

Mean ± SD of three determinations

ePrint@CFTRI

The recovery of protein by alkaline extraction and isoelectric precipitation was reported in the range of 49-56% (Gillberg and Tornell, 1976b; Girault, 1973). Use of alcohol for production of protein isolate from alkali extracted proteins was reported by Keshavarz et al. (1977).

Membrane processes have been used to concentrate, to increase the purity of protein and to remove small molecules (impurities). These processes are effective in removing the glucosinolates and the phytates, as they are relatively small and pass through the pores of the membrane. However, the relatively large phenolic-protein complexes tend to be retain by the membrane along with the protein isolates (Diosady et al., 2003). Washing step before drying would help to maximize removal of anti-nutritional factors. However, practically it is not possible to wash ultrafiltered protein concentrate before drying. Removal of isothiocyanate and oxazolidinethione in all methods were high mainly due to treatment with activated carbon which remove these hydrolysis products, as reported by Woyewoda et al. (1978).

Tzeng et al. (1990) have reported a method for isolation of canola protein including alkaline extraction, isoelectric precipitation, ultrafiltration followed by diafiltration and drying. The protein isolates obtained had high protein content (>90%) low in phytates (<1%) and essentially free of glucosinolates (<2.2 μmol/g). However, the protein isolates were dark in color and had an unpleasant taste. These organoleptic problems were believed to be caused by the presence of phenolic compounds (Sosulski, 1979).

The heat coagulation has been used by number of researcher for the preparation of plant protein concentrate. Protein coagulation was carried out at various temperatures with or without pH

ePrint@CFTRI

adjustment of the protein solution (Xander and Hoover, 1959; Pirie, 1975; Birch et al., 1976; Knorr, 1980). Heat coagulation by steam injection has been applied to recover protein from plant and potato juice (Strolle et al., 1973; Kohler and Knuckles, 1977; Knorr, 1977). There are some reports on the use of heat treatment to recover protein from rapeseed (Thompson, 1977; Mieth et al., 1983b; Kroll et al., 1991). However, there are no available reports on the use of steam injection method to recover protein from mustard/rapeseed.

In addition to recovery of protein, heat treatment has several advantages in removal of anti-nutritional constituents and also to improve nutritional quality of the meal protein (Finnigan and Lewis, 1985; McCurdy, 1990).

Hydrothermal treatment has been proved as an effective method in eliminating split products of glucosinolate (Mustakas et al., 1965; Mieth et al., 1983b). Rachberger et al. (1979) reported different approaches used for eliminating the toxic factors by decomposition of glucosinolates through chemical or enzymic methods. The resulting volatile compounds were removed by steam stripping. Mansour et al. (1993) have reported that the toasting and autoclaving decreased glucosinolates, phytic acid and tannic acid by 47-94%, 9-43% and 41-67%, respectively. Trypsin inhibitor activity was destroyed completely by heat processing. Autoclaving caused more effective reduction in concentration of anti-nutritional factors than toasting.

Duhan et al. (1989) found that autoclaving lowered phytic acid by 20-25 % in chick pea and by 35-40 % in black gram grain. Autoclaving soy isolates at 115° C for 4 h appeared to destroy phytic acid, probably by hydrolysis (O'Dell, 1969).

ePrint@CFTRI

In addition to beneficial effect, it has been shown that heat treatment have some negative effect on rapeseed protein. Excessive heat treatment leads to undesirable millard or browning reaction and reduce the availability of amino acid, especially lysine (Clandinin et al., 1959). Other negative effects of excessive heat treatment were decrease in digestibility and solubility of protein (Wu and Inglett, 1974; Knorr, 1980; Mieth et al., 1983b), formation of some toxic products like lysinoalanine (Maga, 1984; Strenberg et al., 1975; Deng et al., 1990), thermal degradation of glucosinolate (Jensen et al., 1990) and amino acid destruction (Dijkstra et al., 2003). Therefore, it is very important to decrease the time and temperature during heat coagulation to ensure that these negative effects are minimized.

Hence in the present investigation protein solution after carbon treatment was steam injected to rise temperature to 93 ± 2°C for a period of 10min. This condition resulted in maximum recovery of protein with minimum negative effects. The coagulated protein was cooled and separated by centrifugation. The wet protein was washed two times with distilled water in a ratio of 1:5 and separated by centrifugation. The wet protein isolate was dispersed in water (20% solid) and neutralized with acid/alkali and spray dried. The washing step during isolation of protein was very important. A two stage water-leaching process gave mustard protein isolate with low levels of anti-nutritional constituents.

Washing improves the purity and color of protein isolate and eliminates many anti-nutrients (El-Nockrashy et al., 1977). Use of water extraction for removal of anti-nutritional factors has been reported by many investigators. Ballester et al. (1970) have reported that double water extraction resulted in reduction of 84% in VOT and 77% in

ePrint@CFTRI

isothiocyanates. However 2h continuous water extraction procedure with stirring completely removed the isothiocyanates and oxazolidinethione by 97% (Ballester et al., 1973). Cheryan (1980) reported that the soaking of soybean at room temperature and at 55°C for 24 h reduced phytic acid by 50 and 90%, respectively. The leached water contained significant amounts of phytic acid.

8. Enzyme Activation

Among detoxification procedures for removal of glucosinolates, activated carbon and heat treatment were effective in removing glucosinolate hydrolysis products, isothiocyanates and oxazolidine-thione. Therefore, it was necessary to optimize conditions for activation of the enzyme myrosinase which is responsible for hydrolysis of glucosinolates.

Myrosinase is a glycoprotein with molecular weight of about 133 KDa. Its optimal activity takes place at pH values between 4.5 and 8.0 and humidity of more than 13%; ascorbic acid operates as a simulator (Mieth et al., 1983a).VanMegen (1983) reported that incubation at 40°C has an extremely favorable effect on the extraction process of the glucosinolates. In general ascorbic acid exerts an activating effect on plant myrosinase although the extent of this activation is variable. Ohtsuru and Hata (1979) have studied the interaction of myrosinase with ascorbic acid and found that the ascorbic acid is not directly involved in catalysis but changes the conformation of its active site. The presence of ascorbic acid reduces the optimum temperature of myrosinase from 55 to 35°C. Application of crushed mustard seed and ascorbic acid at very low level (0.3g/100g) reduced the time of incubation for complete extraction of glucosinolate from 1h to 30 min (VanMegen, 1983).

ePrint@CFTRI

Therefore based on the results of the above studies, mustard protein isolate was prepared as follows. The meal was dispersed in 1:15 ratio of 0.1M NaCl solution and ascorbic acid (0.1% w/v) was added. The dispersion was incubated at 40°C for 30 min at pH 7.0, then pH was raised to 11 and process followed as discussed in materials and methods (Section II). The efficiency of this method on the removal of toxic and anti-nutritional factors such as glucosinolate hydrolysis products, phytates and phenolics in the isolated protein prepared from dehulled defatted meal and low hull fraction of cake has been discussed in following pages.

A. Isolation and Detoxification of Protein from Dehulled Meal

The chemical composition of dehulled defatted mustard meal and protein isolate prepared from it, are shown in Table 28. The protein isolate prepared from dehulled defatted meal contained 95% protein compared to 48% protein in the starting material. The different steps adopted in the process resulted in a protein with high purity and negligible amounts of other constituents. The yield of protein isolate was 60% (Table 27). The protein isolate was light in color and bland to taste (Fig 30).

Tzeng et al. (1988a) have isolated the protein from rapeseed by membrane processing with a protein content of 90%. The protein isolates prepared from rapeseed by counter current extraction and isoelectric precipitation at pH 6.0 and 3.6 has been shown to contain 90% protein (El-Nockrashy et al., 1977).

ePrint@CFTRI

Table 28. Chemical Composition of Dehulled Defatted Meal and Protein Isolate

Constituents (%)	Dehulled Defatted Meal	Protein Isolate
Moisture	8.0 ± 0.3	5.0 ± 0.5
Fat	1.0 ± 0.2	ND
Protein (N×6.25)	48.0 ± 0.5	95.0 ± 0.5
Ash	5.2 ± 0.2	Trace
Crude Fiber	3.0 ± 0.3	ND
Carbohydrate (by diff.)	35.8 ± 0.5	Trace

ND: Not Detectable

Mean ± SD of three determinations

ePrint@CFTRI

Protein isolate prepared from dehulled defatted canola meal was reported to have a protein content of 72.6% (Thompson et al., 1976). The values were comparable with the reported values. However, the purity of the protein was higher than the reported values.

The presence of anti-nutritional factors in protein isolate as compared to dehulled defatted meal is presented in Table 29. The glucosinolates upon enzymatic hydrolysis are known to produce undesirable and toxic constituents, such as isothiocyanates and oxazolidinethione. The defatted meal contained 18.75 and 13.75 mg/g isothiocyanates and oxazolidinethione, respectively. In the protein isolates, they were reduced to 0.44 mg/g and to a level difficult to detect, respectively. The removal was almost complete compared to the starting material. The reduction of theses constituents mainly was due to activated carbon treatment, steam injection and washing step involved during isolation of protein.

Ballester et al. (1970) have reported that treating the rapeseed cake with steam and water extraction reduced the isothiocyanates and oxazolidinethione content by 77 and 84%, respectively. A two stage process followed by activated carbon treatment reduced the isothiocyanates in rapeseed protein almost completely (Woyewoda et al., 1978). Goering (1963) has developed a process in which ground rapeseed was moistened with cold water or the rapeseed meal was slurried to activate myrosinase and the liberated isothiocyanates were removed by steam stripping. Josefsson (1975) reported that there was a drastic reduction in glucosinolate content when the rapeseed had been heat treated for 1 to 2 h at 115 or 120°C.

ePrint@CFTRI

Table 29. Anti-nutritional Factors Present in Dehulled Defatted Meal and Protein Isolate

Constituents	Dehulled Defatted Meal	Protein Isolate	Removal (%)
Isothiocyanates (mg/g)	18.75 ± 0.3	0.44 ± 0.05	99.5 ± 0.1
5-Vinyloxazolidine-2-thione (mg/g)	13.75 ± 0.35	ND	100
Phytic acid (%)	5.6 ± 0.1	0.25 ± 0.03	99.0 ± 0.3
Phenolics (%)	2.12 ± 0.06	0.12 ± 0.02	98.7 ± 0.3
Trypsin Inhibitor Activity (TIU/mg Protein)	3.8 ± 0.1	ND	100

ND: Not Detectable

Mean ± SD of three determinations

ePrint@CFTRI

Zeb et al. (2002) reported that heating with steam for 30 and 60 min reduced the glucosinolates and sinapine content in the meal. Protein concentrate prepared from different varieties of rapeseed meal by water leaching removed the isothiocyanates and goitrin by more than 90% (Jones, 1979). Ismond and Welsh (1992) have reported that protein isolates from canola meal by PMM method had very low glucosinolate content.

The phenolics and phytic acid content of defatted meal were 2.12% and 5.6%, respectively. In the protein isolate, they were reduced to 0.12 and 0.25%, respectively (Table 29). The reduction of phenolics and phytic acid was 99% on the basis of protein yield. The removal of phytic acid was due to extraction of protein at pH 11 (where the solubility of phytic acid is minimum), activated carbon treatment, use of NaCl, heat coagulation and washing. Reduction of phenolics may be due to activated carbon treatment, heat treatment, washing, use of NaCl and also probably due to the use of ascorbic acid as an anti-oxidant.

Activated carbon treatment has been effectively used in the removal of glucosinolate and phenolic compounds in rapeseed protein (Woyewoda et al., 1978; How and Morr, 1982). Tzeng et al. (1988a) have reported that extraction of protein with sodium hexametaphosphate solution followed by activated carbon treatment, ultrafiltration, diafiltration and purification by ion-exchange resulted in complete removal of glucosinolate and reduced phytate. The resulted protein was light in color and bland to taste. Activated carbon treatment removes phenolic compounds by physical adsorption, e.g. dipole-dipole interaction, hydrogen bonding; Van der Waals and similar forces as in ionic and covalent bonds (Garten and Weiss,

ePrint@CFTRI

1957). Seo and Morr (1985) reported that ion-exchange and activated carbon treatment removed 92 and 82% of the total phenolic acids from peanut protein isolates, respectively.

Ismond and Welsh (1992) have reported that extraction of canola protein at pH 5.5 in the presence of 0.1 M NaCl reduced phytic acid and phenolics by 75% and 85%, respectively. Xu and Diosady (2000) found that some 50% of the extracted phenolic compounds formed complexes with canola proteins by several distinct mechanisms among which ionic binding was predominant accounting for about 30%. The phenolic fractions bound by hydrophobic interactions, hydrogen bonding and covalent bonding were relatively small, each constituting less than 10% of the total extractable phenolics. The quantitative distribution of phenolic-protein complexes indicated that treatment with 0.05M NaCl could break phenolic-protein complexes bonded ionically, which represent some 30% of the total phenolic acids present in the extract. The presence of salt lowered the amount of phytic acid bound to the protein (Mothes et al., 1990). Diosady et al. (2003) have reported that use of ascorbic acid or sodium sulfite at low concentration during alkaline extraction of protein partially inhibit the formation of covalently bounded phenolic-protein complex and reduce the overall concentration of residual phenolic compounds in the protein isolate. Presence of reducing agents has been shown to be effective in protecting protein from lysinoalanine (LAL) formation (Finley and Kohler, 1979).

The trypsin inhibitor activity in dehulled defatted meal was 3.8 TIU/mg protein and it was completely inactivated in the protein isolate. This may be due to heat treatment used for recovery of protein.

ePrint@CFTRI

Mansour et al. (1993) reported that trypsin inhibitor activity in rapeseed was totally inactivated by toasting and autoclaving.

B. Isolation and Detoxification of Protein from Commercial Cake

In India, mustard seed is processed for oil by crushing the whole seed with Ghani/expeller pressing and cake obtained contains high amount of crude fiber and other anti-nutritional constituents being used as animal feed or fertilizer. Therefore, in order to reduce the fiber content, the fractionation of mustard cake has been used to reduce the hull content up to 50% (Fig 9, section II). The low hull fraction was used in the preparation of protein concentrate free of toxic and anti-nutritional factors. The results are discussed below.

The chemical composition of low hull fraction and protein concentrate prepared with this fraction presented in Table 30. The protein content of isolated protein from cake was lower than the protein isolated from dehulled defatted meal. Generally, a protein isolate must not contain less than 90% protein (N×6.25) on moisture free basis**.** Probably the lower protein content of isolated protein (concentrate) from low hull fraction may be due to lower protein content of this fraction (40.5%) compared to mustard meal (48%). Also, the increased temperature during crushing of seed (due to mechanical abrasion) may denature protein which results in lower protein solubility in cake compared to defatted meal. In addition protein may get modify by the reaction with glucosinolate hydrolysis products, like isothiocyanates, during crushing of seeds (Dijkstra et al., 2003).

ePrint@CFTRI

Table 30. Chemical Composition of Low Hull Fraction of Cake and Protein Concentrate

Constituents (%)	Low Hull Fraction of Cake	Protein Concentrate
Moisture	8.9 ± 0.1	7 ± 0.5
Protein (N×6.25)	40.5 ± 0.5	80 ± 1.0
Fat	6.1 ± 0.1	3.8 ± 0.2
Ash	2.2 ± 0.1	ND
Crude Fiber	5.4 ± 0.1	0.5 ± 0.1
Carbohydrate (by diff.)	36.7 ± 0.2	8.7 ± 0.5

ND: Not Detectable

Mean ± SD of three determinations

ePrint@CFTRI

The protein solubility profile of mustard cake (low hull fraction) at different pH is presented in Figure 29. In general, the solubility profile at all the pH was lower compared to dehulled defatted meal (Fig 27). It showed two solubility minima at pH values of 4 and 8.2. The difference in solubility minima between dehulled defatted meal and low hull fraction of commercial cake may be due to varietal difference and/or effect of heat during crushing on the protein structure. However under alkaline condition (pH 11) a maximum of 75% nitrogen could be extracted (Fig 29).

The anti-nutritional factors presented in low hull fraction and protein concentrate are presented in Table 31. 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 concentrate, 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 concentrate, 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 3.5 TIU/mg of protein and completely inactivated in protein concentrates. The results were similar to those observed in the case of protein isolate from dehulled defatted meal. The protein concentrate was light yellowish in color and bland to taste with low content of anti-nutritional factors (Fig 31). The bland light colored protein with reduced anti-nutritional factors could be used in various food formulations.

ePrint@CFTRI

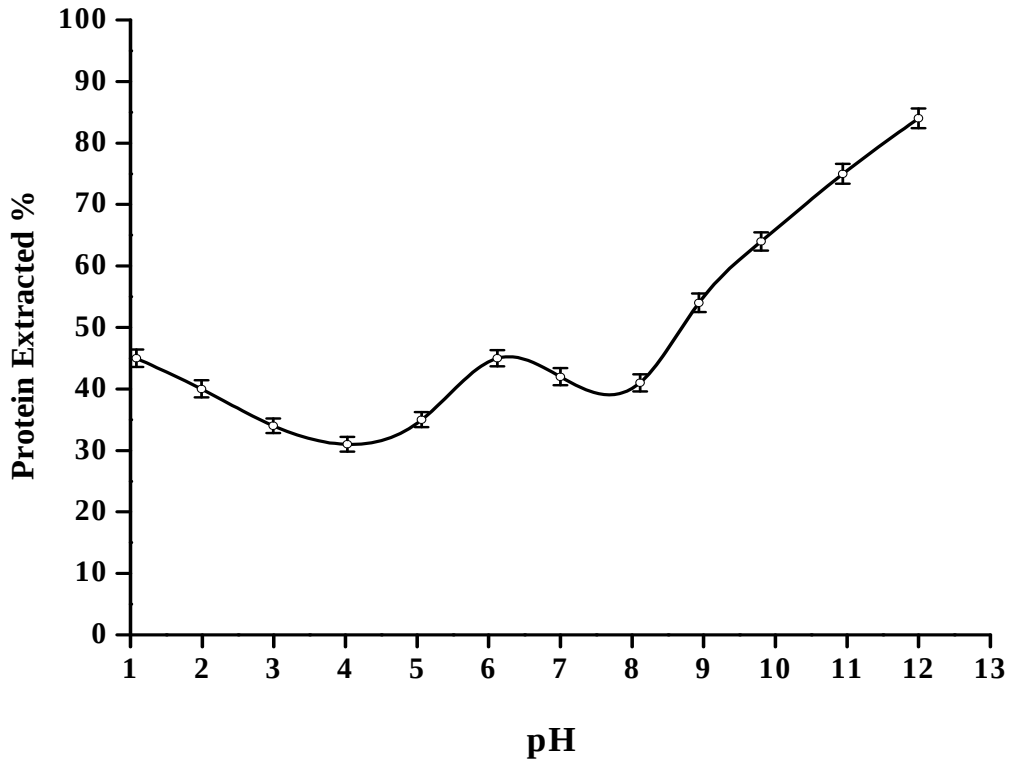


Fig 29. Protein Solubility Profile of Low Hull Fraction of Cake at Different pH Values

ePrint@CFTRI

Table 31. Anti-nutritional Factors Present in Low Hull Fraction of Cake and Protein Concentrate

Constituents	Low Hull Fraction of Cake	Protein Concentrate	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

ND: Not Detectable

Mean ± SD of three determinations

ePrint@CFTRI

Fig 30. Protein Isolate Prepared from Dehulled Defatted Mustard Meal

Fig 31. Protein Concentrate Prepared from Low Hull Fraction of Commercial Cake

ePrint@CFTRI

Detailed knowledge of structural and physical characteristics of protein is essential for understanding and manipulation of their properties in foods (Kinsella, 1976). It is well known that the processing methods and conditions change the physicochemical properties of protein. In this investigation, the protein isolation was involved steam injection and enzyme modification which may change the physicochemical properties of the protein. Since there was not much information available on these aspects, therefore the effect of these processing methods on the proteins by SDS-PAGE and scanning electron microscopy (SEM) has been studied. The color characteristics of the isolated protein and the starting materials were also evaluated by Hunter color measurement system.

Functional properties are important in determining the usefulness of protein in food systems. The functional properties of proteins play an important role in the product development (Kinsella, 1976). In this chapter, the selected functional properties of isolated mustard protein products (protein isolate/concentrate and hydrolysates) and starting materials (meal/cake) compared to defatted soybean meal were studied. The chemical composition of soybean meal used in this study is given in Table 32. The method used to recover protein may cause changes in the functional properties. Therefore, study on functional properties may provide useful information about these changes. Such information may be helpful in the use of mustard protein isolate in formulation of new high protein foods. The functional properties studied were nitrogen solubility, water absorption capacity, fat absorption capacity, bulk density, foam capacity, foam stability and emulsion capacity.

ePrint@CFTRI

Table 32. Chemical Composition of Soybean Meal

Constituents	Amount (%)
Protein (N×6.25)	52 ± 1
Fat	2.5 ± 0.2
Ash	6.5 ± 0.1
Crude Fiber	3.5 ± 0.1
Moisture	7 ± 0.5
Carbohydrate (by diff.)	28.0 ± 1

.

Mean ± SD of three determinations

ePrint@CFTRI

Color

The color of mustard/rapeseed protein products is frequently poor and limits the use of these products in foods. It is well-known that undesirable grayish brown coloration, often found in protein isolate derived from oilseed is mainly due to phenolics, which during processing readily oxidize to quinonoid substances that become irreversibly bound to protein. Phenolics compounds on oxidation can cause the development of dark colors in oilseed protein products. Under alkaline conditions they readily undergo enzymatic and non-enzymatic oxidation to form quinones which can then react with protein, resulting in dark green or brown color in the protein solutions and when the proteins are precipitated at their isoelectric points, the color cannot be washed from the protein isolates (Sosulski, 1979).

In this study, different treatments were used in order to produce a protein isolate with acceptable color characteristics. These treatments include activated carbon treatment, use of NaCl to break the phenolic protein interaction, use of ascorbic acid as an antioxidant and washing of protein isolates to remove chlorogenic compounds. The effect of these treatments on the color characteristics of isolated protein products and starting material were determined using a hunter colorimeter. The results of color measurement of meal, protein isolate and hydrolysate prepared from meal, low hull fraction of cake, protein concentrate and hydrolysates prepared from this fraction are presented in Figures 32 to 35.

For objective color measurement of food, color scales are used to measure color and its differences between products. Color is often defined using three dimensional color scales that describe the

ePrint@CFTRI

different component of color. Light reflected from a color object is composed of a light or dark component in addition to a red or green and a blue or yellow component. A Hunter colorimeter measures the color of samples in the three-dimensional opponent-color system. All *L* values are measures of sample lightness, with 100 being white and 0 black. All *a* values indicate redness varying between +100 and -80 as sample color change from red to green, whereas yellowness measured by *b* values from +100 (yellow) to -80 (blue) (Mabon, 1993; Xu and Diosady, 2002).

The results of *L* value of different samples are presented in Figure 32. The results showed that the *L* values (lightness) of the protein isolate and hydrolysate were 69.8 and 69.3 compared to 76.5 for dehulled defatted meal. The low hull fraction of cake that had slightly dark color showed *L* value of 54.6. The lightness was improved after the isolation and detoxification in the concentrate and hydrolysate (Fig 32). The results clearly indicate the correlation between removal of phenolics and improvement of sample lightness. The lower *L* values of protein isolates and hydrolysate could be due to covalently bonded phenolic-protein complexes formed during processing which can not be removed easily. The low hull fraction of cake showed the *L* value of 54.7 compared to 76.5 in meal. This low *L* value may be due to the negative effect of crushing of whole seed during the preparation of commercial cake. The darkening of cake during crushing of seed may be due to enzymatic and non-enzymatic browning, oxidation of quinines, etc.

ePrint@CFTRI

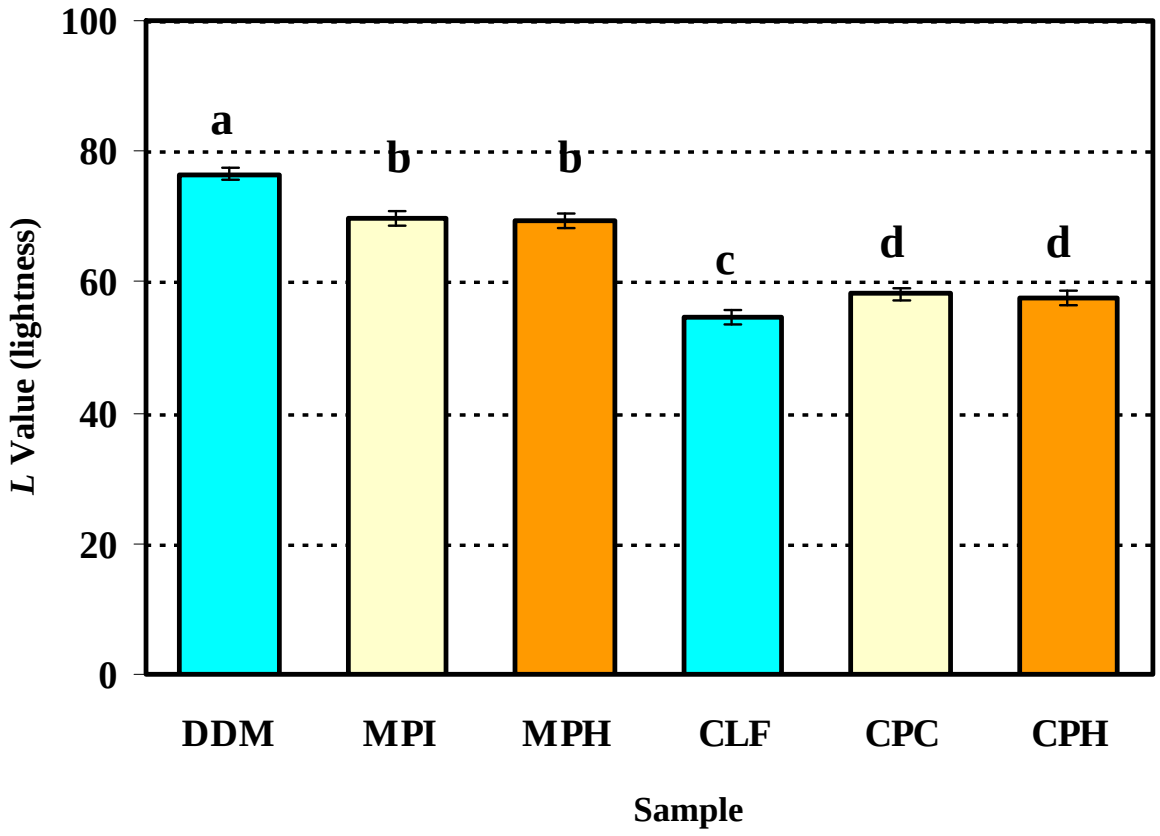


Fig 32. *L* Values of Different Samples

DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

Protein concentrate and hydrolysate prepared from low hull fraction of cake showed higher *L* values compared to this fraction (Fig 32) and this could be the effect of different steps involved in isolation and detoxification including activated carbon treatment, use of NaCl to break ionically-bonded phenolic-protein complexes, use of ascorbic acid as an antioxidant and washing of extracted protein.

Xu and Diosady (2002) studied a variety of treatment for the removal of phenolic compounds from canola protein, to improve the color of protein isolate and reported the *L* values in the range of 52-69 for different treatments. However no treatment could completely eliminated the color and all sample had a light brown color. This was probably because of the covalently bonded phenolic protein complexes, which could not be removed by any treatment. According to mechanism proposed by Torchinskii and Dixon (1974), covalent bonds could be formed through the reaction of SH-containing amino acids such as cysteine with quinones that were derived from phenolic compounds.

The results of *b values* (yellowness) are given in Figure 33. The intensity of yellow color decreased after processing of the meal or cake to protein isolate/concentrate and hydrolysates. The intensity of yellow color was higher in meal and its protein products than the cake and its protein products. Xu and Diosady (2002) reported the *b* values of rapeseed protein isolate treated with different methods in the range of 12.9 to 17.5.

The results of *a* values (Fig 34) showed decrease in redness (or increase in greenness) of samples after isolation and detoxification.

ePrint@CFTRI

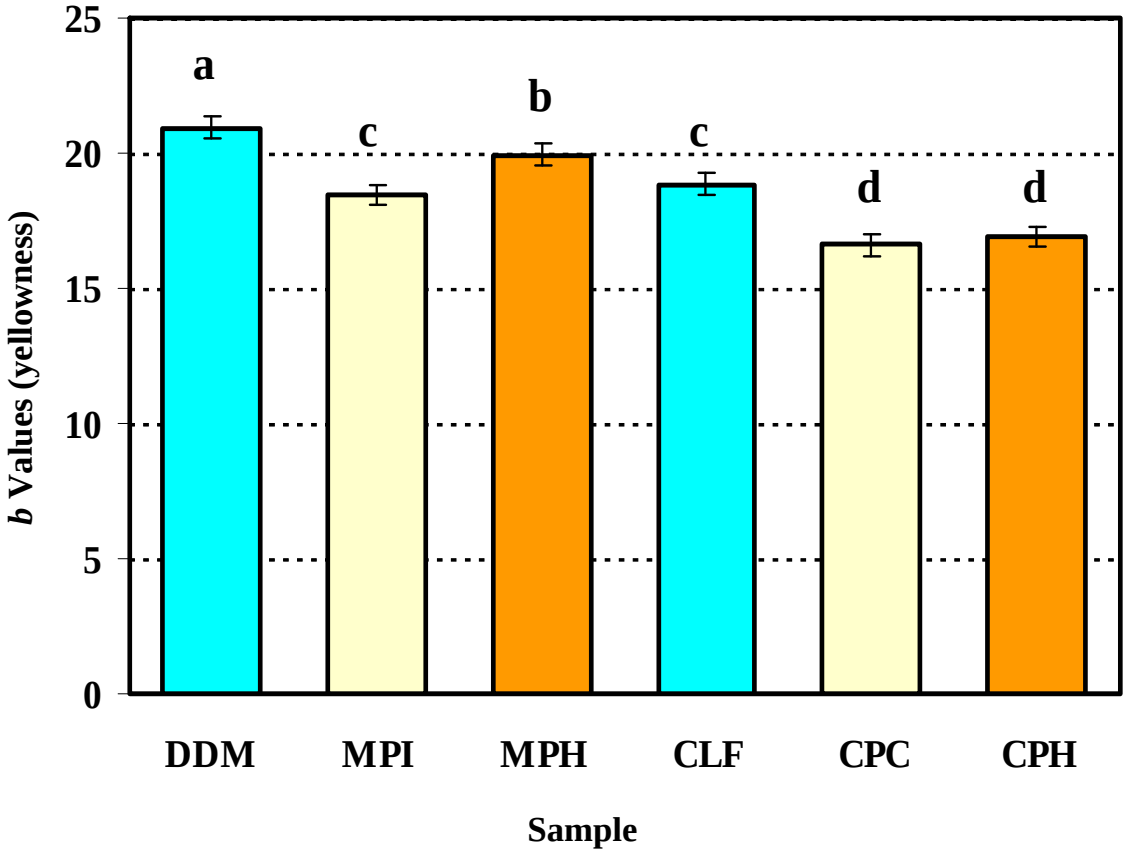


Fig 33. *b* Values of Different Samples

DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

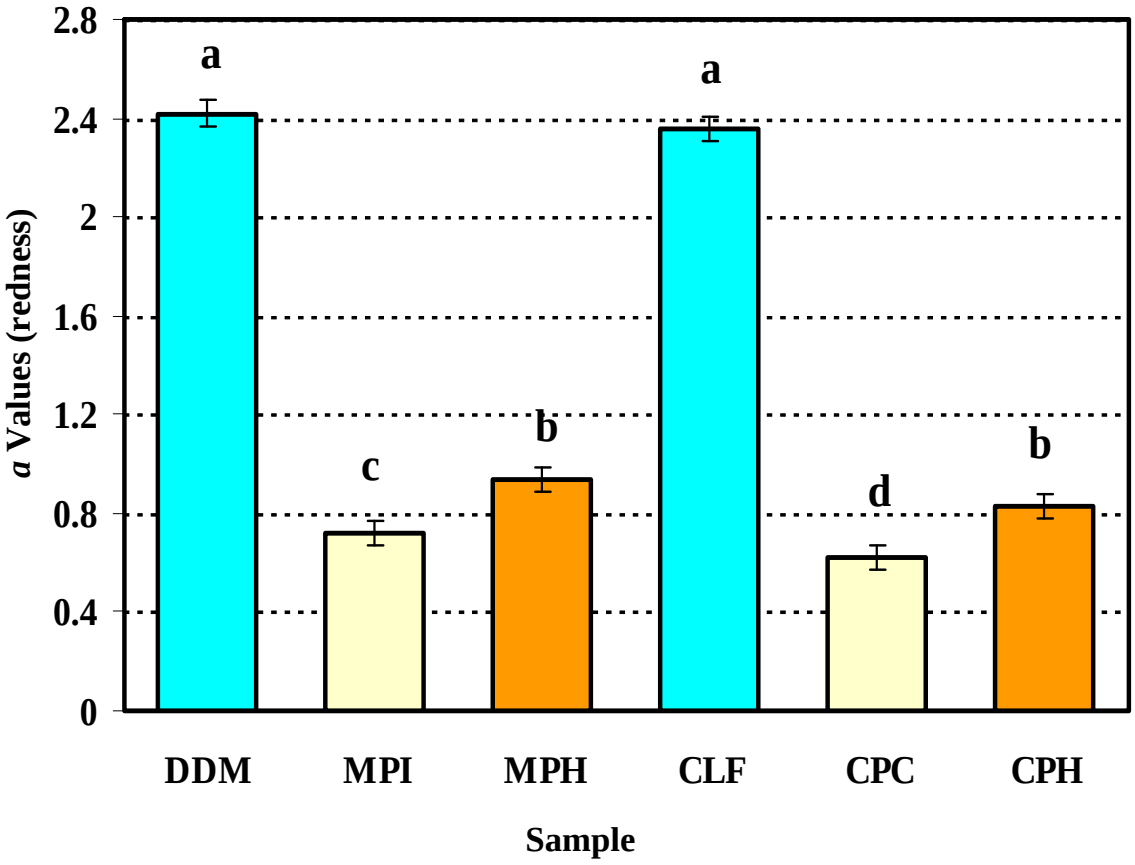


Fig 34. *a* Values of Different Samples

DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

The increase in greenness may be due to effect of alkaline treatment used for extraction of protein (Fig 34).

A dark brownish or grayish color which usually develops in protein extracted from rapeseed meal, particularly under alkaline conditions, has been reported by others (Appleqvist, 1971; Keshavarz et al., 1977; Sosulski, 1979; Thompson et al., 1982a). Gillberg and Tornell (1976a, 1976b) extracted the rapeseed protein under high alkaline conditions (pH 11) without simultaneous extraction of phytic acid. However the product was brown and remained brown even after alcohol treatment because of large amount of phenolics that were co-extracted with the proteins at high pH (Thompson et al., 1982a).

Polyphenolic compounds in plants can bind with protein, reversibly by hydrogen bonding and irreversibly by oxidation followed by covalent condensation (Loomis and Battaile, 1966). It is possible that the products of protein-phenolic interaction are involved in the formation of the green and brown color of the rapeseed protein products (Lo and Hill, 1972). Xu and Diosady (2002) reported that the dark color of canola protein isolates is dependent on their phenolics content and treatment that removes phenolic compounds lead to lighter colored protein isolates.

Diosady et al. (2003) reported that the oxidation of phenolic compounds under alkaline conditions increased the covalent binding of phenolics to protein, hence darkening the color of protein extracts or solutions. Therefore, the addition of the anti-oxidant at least partially inhibits the formation of covalently bonded phenolic-protein complexes, thus reducing the overall concentration of residual

ePrint@CFTRI

phenolic compounds in the isolates. The anti-oxidants that they used were sodium sulfite (Na_2So_3) and/or ascorbic acid.

Therefore, the effect of ascorbic acid on color characteristics of protein isolate was studied. Protein isolate was prepared with ascorbic acid and without ascorbic acid and the data on color characteristics of protein isolates are shown in Figure 35. The results showed that the use of ascorbic acid in the process resulted in a protein isolate with higher color characteristics. The results are in good agreement with the findings reported by others (Finley and Kohler, 1979; Diosady et al., 2003).

Scanning Electron Microscopy (SEM)

Traditionally, the structure of food proteins has been studied using light microscopy. The development of electron microscopy enabled the researcher to study the food proteins structure and their interaction with other components of foods. Now the scanning electron microscopy is being used by the food scientists to obtain microstructural information. The technique also is used to study the surface and internal structure, depending upon the preparation methods.

The major proteins of rapeseed are located in sub-cellular packages called aleurone grains that are considered to have storage function as opposed to structural or catalytic functions (Appleqvist and Ohlson, 1972). The aleurone grains are membrane-bound within the seed (Gill and Tung, 1976). Commercially prepared meal contains intact, as well as disrupted aleurone grains (Stanley et al., 1976).

ePrint@CFTRI

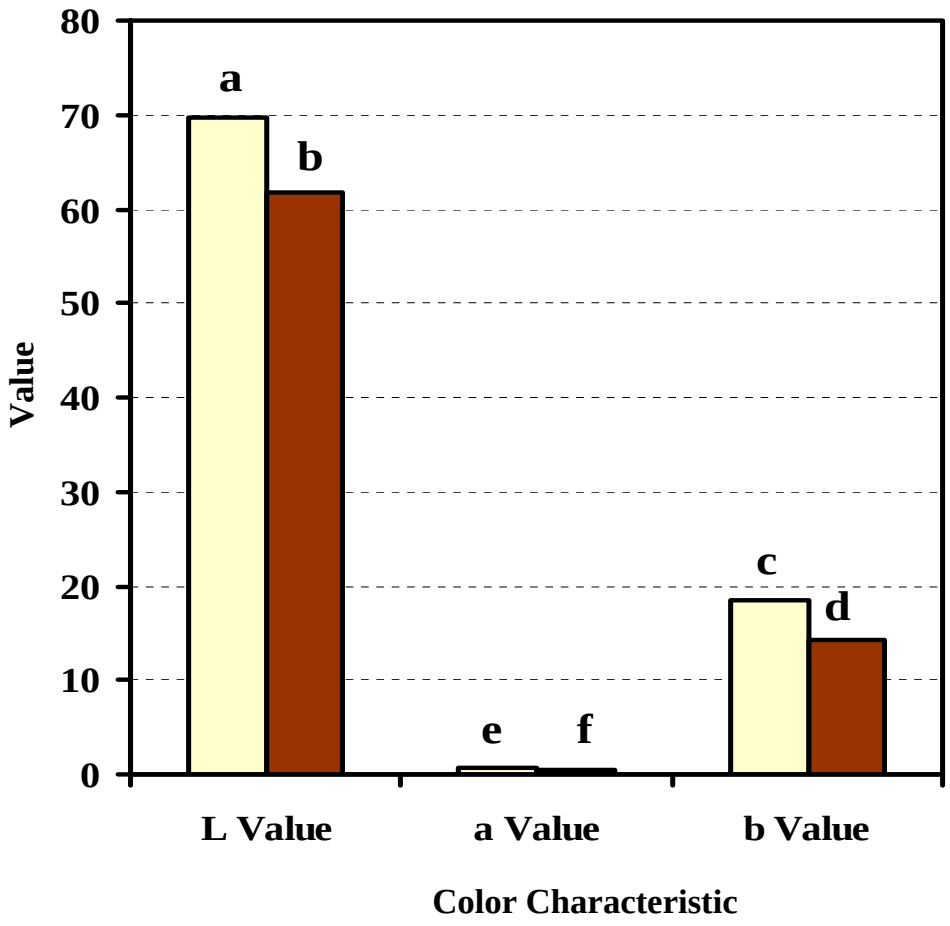


Fig 35. Color Characteristics of Protein Isolate Prepared

With Ascorbic Acid **Without Ascorbic Acid**

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

In the present investigation, effect of processing and isolation methods on the structure of mustard protein has been studied by SEM. The SEMs of meal and low hull fraction of cake are presented in Figure 36 and 37, respectively. The spherical particles in these figures seem to be the protein bodies. The amorphous materials that present in these figures probably are protein released by rupturing of the larger protein bodies. The SEMs of protein isolate prepared from meal and protein concentrate prepared from low hull fraction of cake are presented in Figure 38 and 39. The isolated protein contain mostly spherical particles 1-20 μm in diameter probably contained the protein bodies and smaller particles seems to be due to rupture of larger protein bodies during isolation process. The reduction in the size of protein bodies in protein isolate and concentrate compared to meal and cake may be due to the effect of heat treatment.

The SEM of protein hydrolysates prepared from meal and low hull fraction of cake are presented in Figure 40 and 41. The particle size of protein hydrolysates was around 1-10 μm and majority of them were less than 5 μm in diameter. The reduction in size of protein particles was due to enzyme hydrolysis. Vioque et al. (1999) have reported that the most evident change observed in hydrolysates compared to the protein isolates was the reduction in the molecular weight of proteins as a direct consequence of protease activity.

Wolf (1970) reported that protein bodies prepared from defatted soybean flour contained numerous spherical particles 1-3 μm in diameter plus amorphous material, when examined in a scanning electron microscope. The full-fat and defatted soybean flours contained particles 1-10 μm in diameter. The larger protein bodies consequently disrupted during isolation. The flour contained particles which were

ePrint@CFTRI

considerably larger than those presented in the isolated protein. Therefore, the large protein bodies apparently did not survive the isolation process. The amorphous material seen in the protein body preparation may be protein released by rupture of the larger protein bodies. Wolf and Baker (1975) have reported that the soy flour prepared by pin-milling, defatting and screening contained numerous particles in the size range (1-20 μm diameter) expected for protein bodies. The isolated protein bodies are only 1 to 3 μm in diameter, whereas the soy flour contains numerous particles greater than 5 μm in diameter. The SEM of soy flours which heat treated by different methods showed that the greater the extent of heat treatment, the higher the degree of disruption of protein bodies.

ePrint@CFTRI

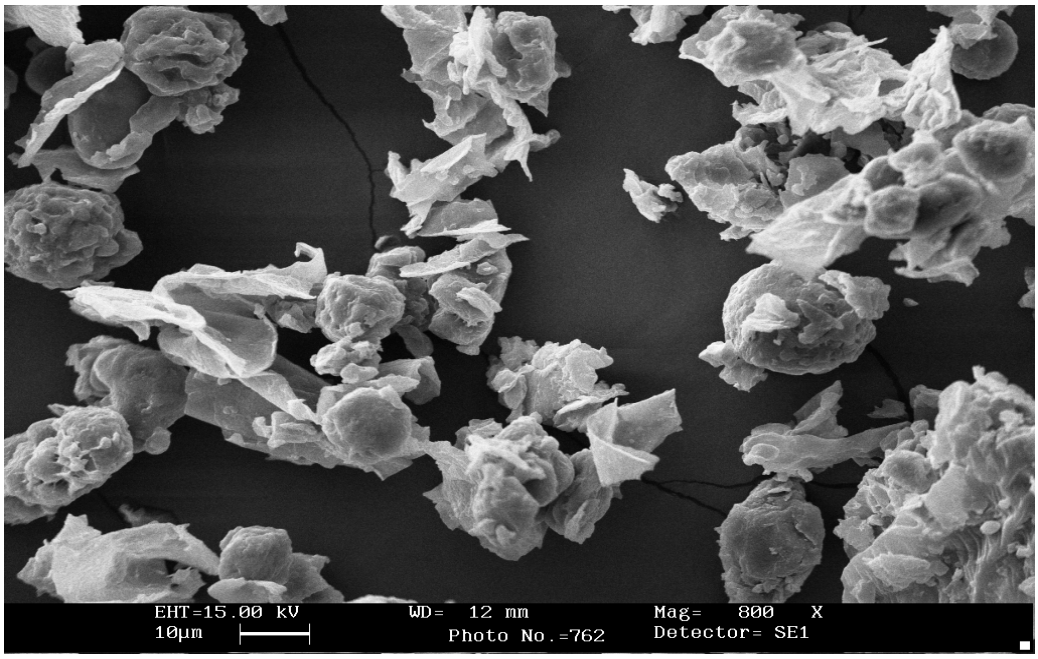


Fig 36. SEM of Mustard Dehulled Defatted Meal

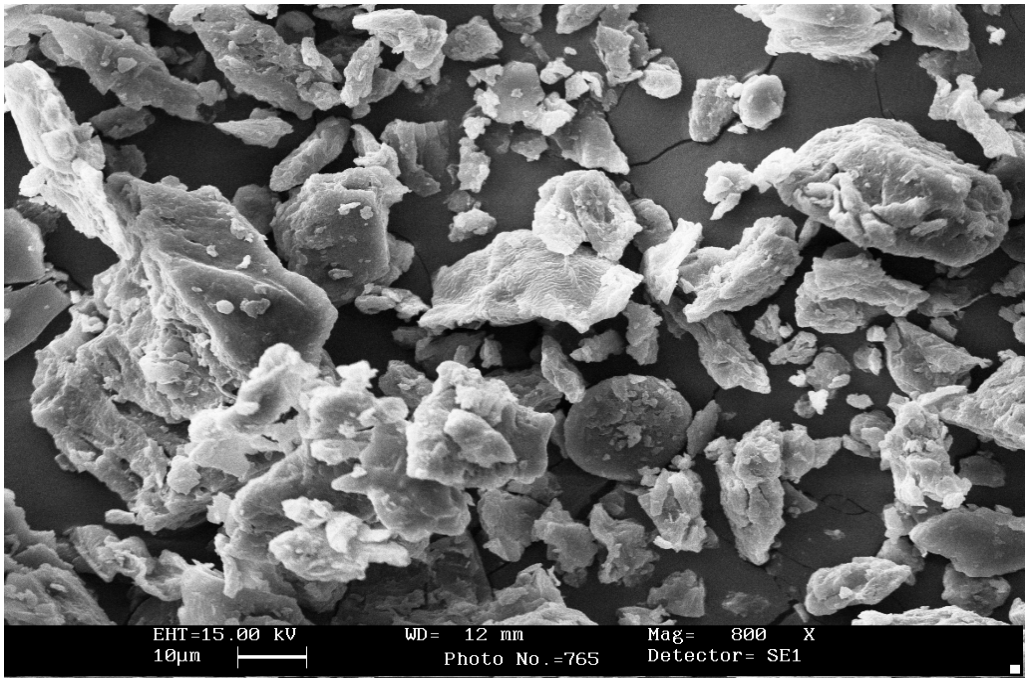


Fig 37. SEM of Low Hull Fraction of Commercial Cake

ePrint@CFTRI

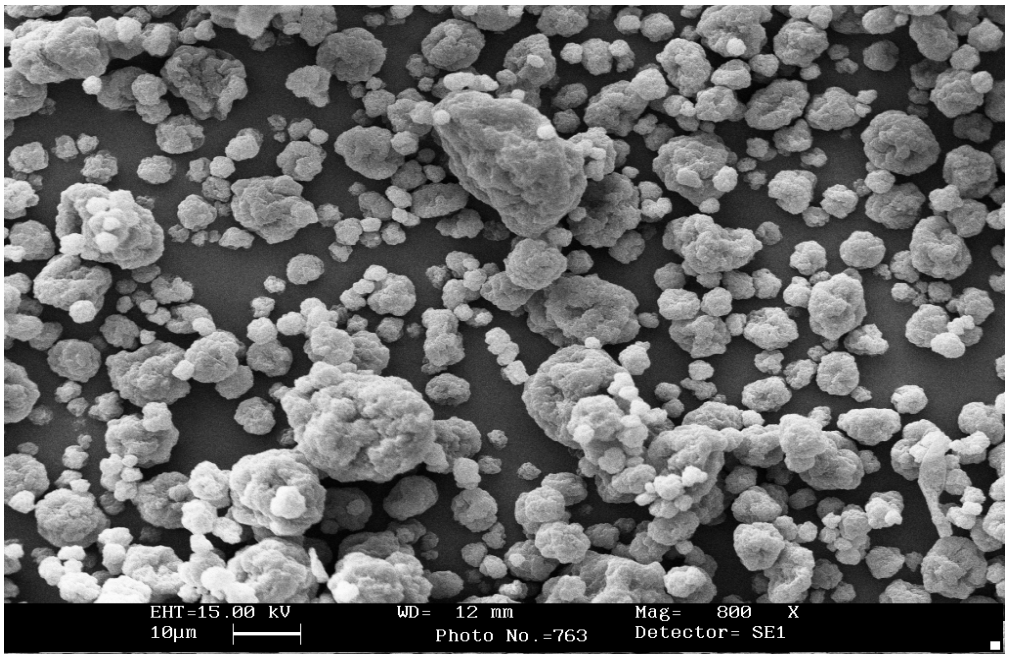


Fig 38. SEM of Meal Protein Isolate

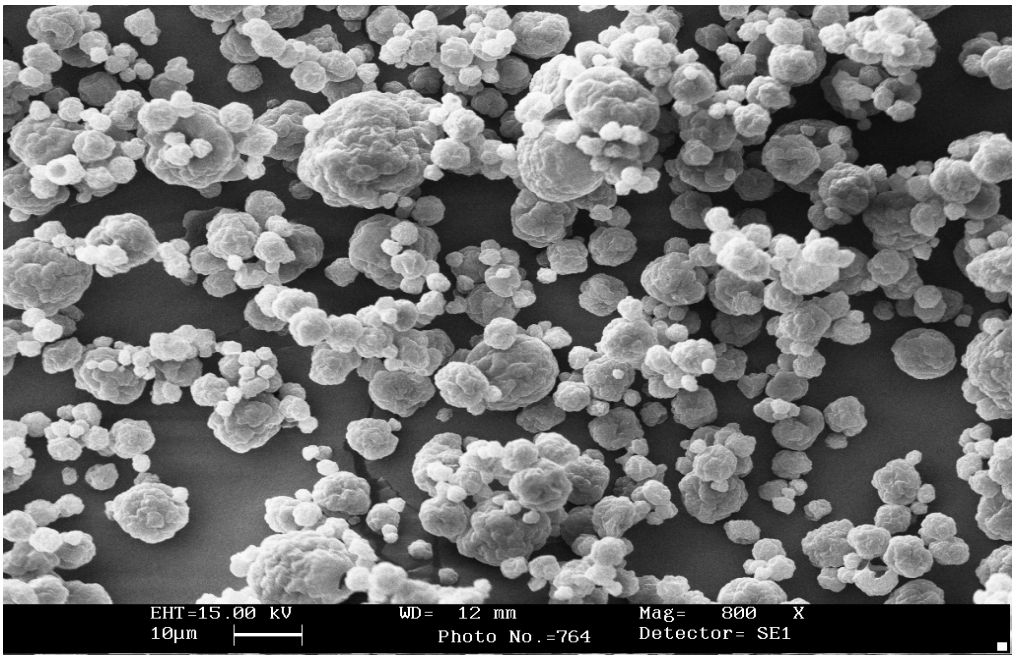


Fig 39. SEM of Cake Protein Concentrate

ePrint@CFTRI

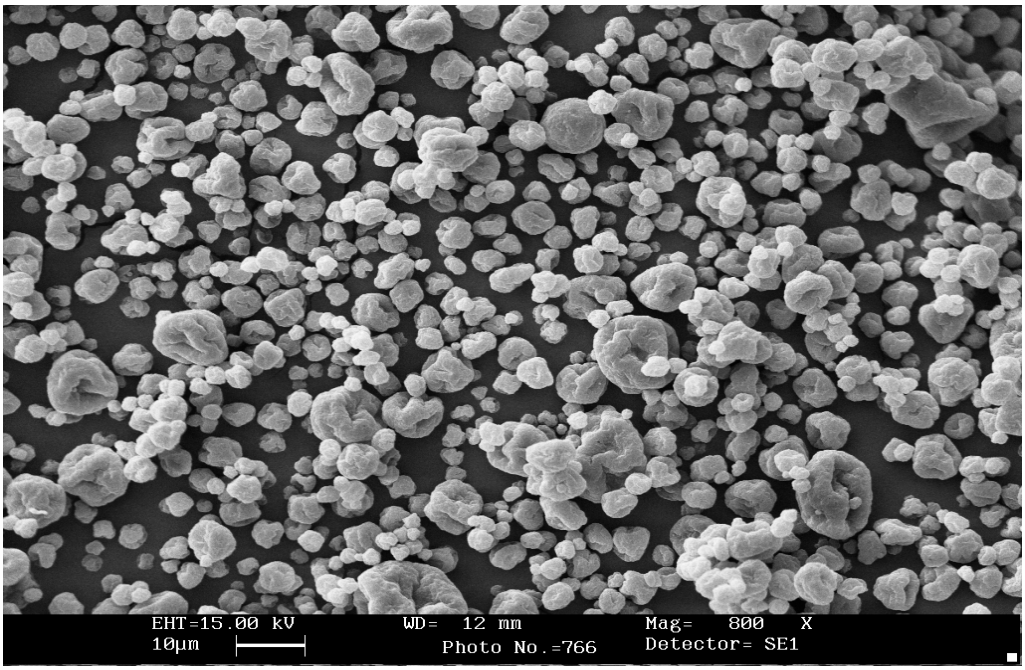


Fig 40. SEM of Meal Protein Hydrolysate

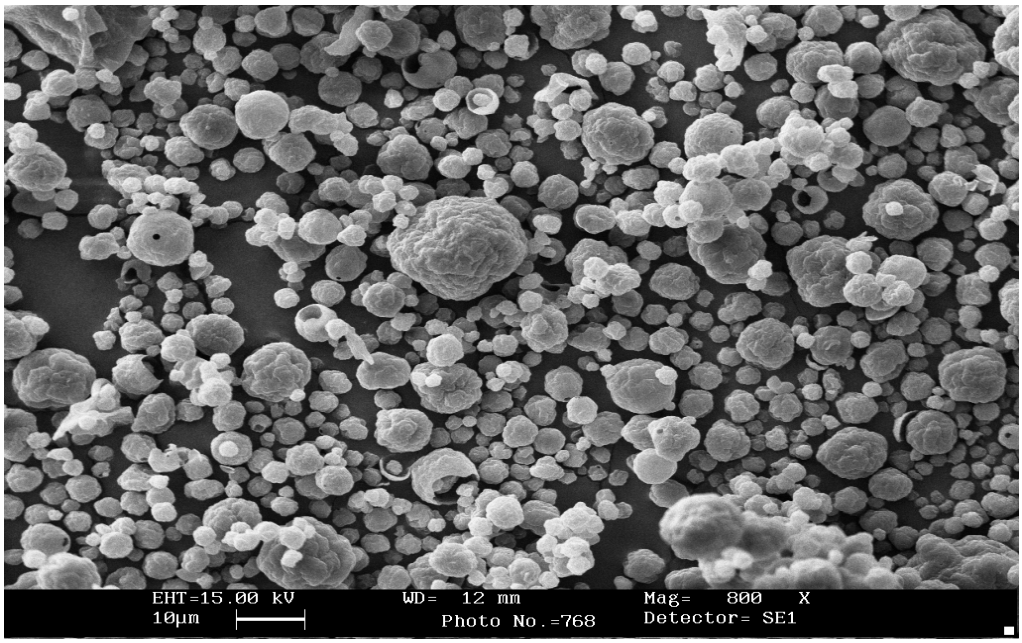


Fig 41. SEM of Cake Protein Hydrolysate

ePrint@CFTRI

Solubility

Solubility behavior provides a good index of the potential (and limitations of) applications of proteins and also gives information useful in the optimization of processing procedures and in determining effects of heat treatments which might affect their actual and potential applications. Nitrogen solubility profile, over a range of pH values, is being used increasingly as a guide to protein functionality, since this relates directly to many important properties, e.g., use in beverages, emulsifications, foaming capacity and gelation. Solubility is affected by a multitude of factors, viz., protein source, processing history, minor and major treatment in preparation and processing, heating, conditions of solubility determination (i.e. pH, temperature, ion types, and their concentration), protein concentration and the presence of other ingredients.

The protein solubility profiles of isolated protein prepared from dehulled defatted meal by different recovery methods are presented in Figure 42. Among the methods used for recovery of proteins, heat coagulation by steam injection resulted in better yield with maximum removal of anti-nutritional factors (Chapter 2). However the use of heat coagulation for recovery of protein resulted in a protein isolate with a lower solubility (20-40%) at different pH compared to other methods. At pH 12 more than 85% of protein was soluble in the case of ultrafiltration, alcoholic precipitation and isoelectric precipitation methods. The protein solubility profile of protein concentrate prepared from low hull fraction of cake is presented in Figure 43.

ePrint@CFTRI

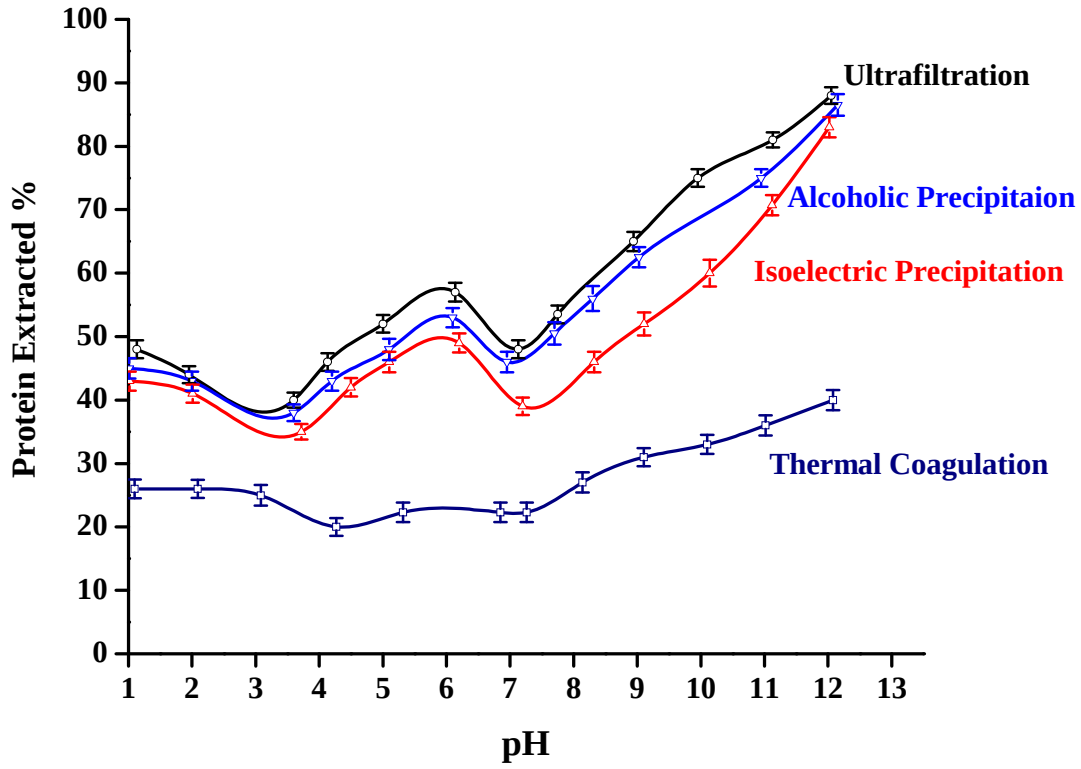


Fig 42. Solubility Profile of Isolated Proteins from Dehulled Defatted Meal Recovered by Different Methods at Different pH Values

ePrint@CFTRI

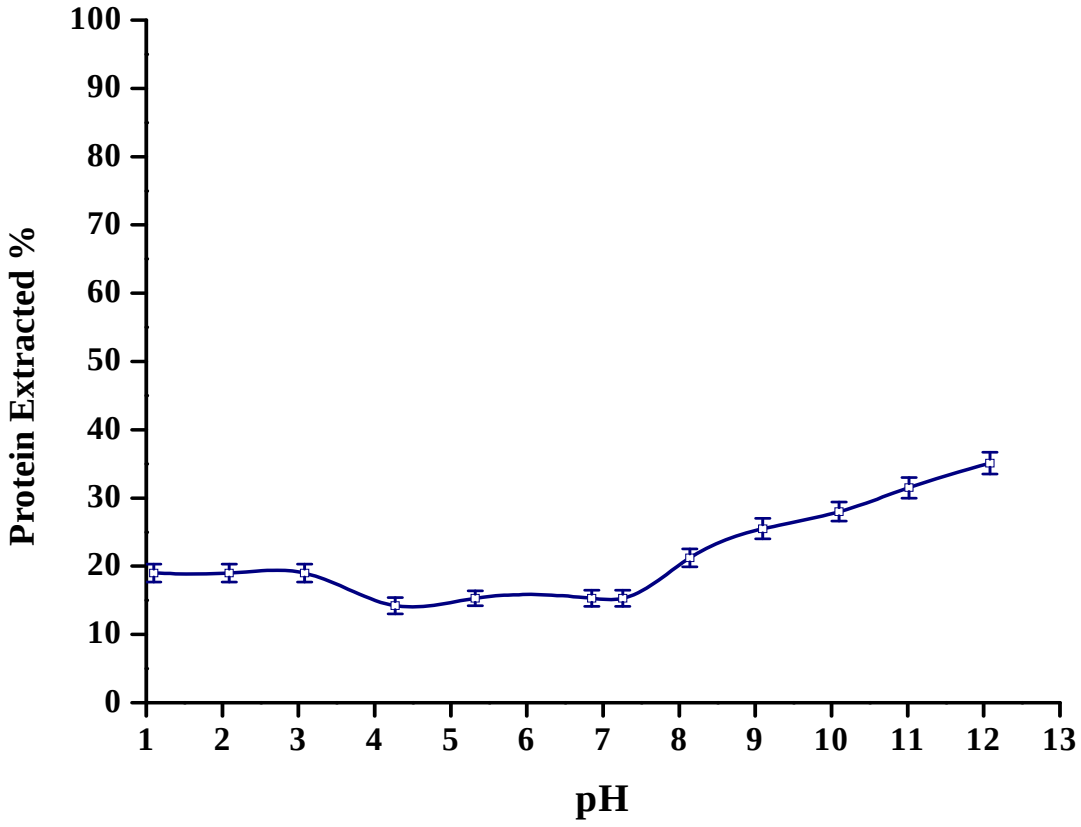


Fig 43. Solubility Profile of Protein Concentrate from Commercial Cake at Different pH Values

ePrint@CFTRI

The protein concentrate showed low solubility of 15-35% at all pH values. The solubility pattern was similar to that of heat coagulated protein isolate prepared from dehulled meal (Fig 42).

Heat precipitation of proteins from extractant causes irreversible denaturation in most instances, and it results in reduced solubility; consequently, many functional properties are destroyed or impaired by heat treatment (Rakesh and Metz, 1973; Betschart, 1974). Rutkowski (1971) has reported that the toasting the rapeseed/canola at 120°C and above decreased the solubility and destroyed more than 50% of the isothiocyanate and oxazolidinethione contents in the meal. More drastic heat treatment resulted in greater destruction of glucosinolates and decreased the solubility of proteins proportionately.

Heat used in the commercial processing of seed products can improve protein quality by destroying certain anti-nutritional factors but concomitantly reduces protein solubility in proportion to the degree of heat treatment (McWatters and Cherry, 1977). Finnigan and Lewis (1985) have shown that heating meal to inactivate myrosinase (cooker-prepress) decreased nitrogen solubility compared to unheated rapeseed meal. Heating the dispersion of rapeseed protein isolate prepared by alkaline extraction and acid precipitation at temperatures of 105°C or higher decreased the nitrogen solubility from 24% to 12% (McCurdy, 1990). Rachberger et al. (1979) have showed the effect of steaming of whole rapeseeds on the protein solubility of defatted meal. They reported that solubility in water at 25°C was about 70% in meal prepared from unheated seeds and decreased to 15% on steaming for 30 min. The decrease in protein solubility was rapid in the initial period of heat treatment.

ePrint@CFTRI

Knorr (1980) studied the effects of different methods of protein coagulation at 22-24°C in comparison with protein coagulation at 98-99°C on protein solubility of spray dried potato protein concentrates. Results indicated that the use of citric acid or ferric chloride at 22-24°C as coagulant resulted in higher protein solubility of 85 to 92% as compared to 12% for the heat coagulated samples.

McWatters and Cherry (1983) reported that heat processing of ground nut flour reduced the protein solubility. Soy flakes subjected to both dry heat (130°C) and wet heat (steaming at 100°C) for different times showed reduced protein solubility (Wu and Inglett, 1974). Heat treatment had deterious effects on the solubility and other functional properties but in the case of soy protein, it had beneficial effect in reducing the beany flavor (Wolf and Cowan, 1975).

Enzymatic Modification of Protein to Improve the Solubility

Vegetable and oilseed constitutes an enormous source of protein for human consumption. However, to be exploited successfully, these proteins must be presented to the consumer in forms that are attractive and possess the flavor, texture and quality desired by the consumer. In view of the multiplicity and variety of food consumed by different ethnic groups worldwide, vegetable proteins ideally should display a spectrum of versatile properties to be suitable for a wide range of applications depending on the functional properties.

Denaturation of protein is of great practical value in food processing and is particularly important in the case of proteins used as functional ingredients. Generally, denaturation is viewed in a negative sense because it usually implies loss of some important functionalities. However, in some instances, denaturation has beneficial effect by

ePrint@CFTRI

destructing the anti-nutritive agents and deteriorative enzymes and enhances the digestibility of proteins. Development of appropriate methods to modify theses non-functional proteins, tend to impart required functional attributes. Successful modification to improve functional properties may also extend the application of novel proteins (Altschul and Wilcke, 1985).

Modification of food proteins is not new.Proteins affect many physical properties of foods. Hence, alteration of proteins by physical or chemical means has provided a resource for designing functional proteins and fabricating new foods. Protein modification usually refers to the intentional alteration of protein structure by physical, enzymatic or chemical agents, to improve functional properties. Currently, interest and research concerned with chemical and enzymatic modification of proteins, particularly novel proteins, to improve their functional characteristics is burgeoning. These improvements can significantly expand the range of uses for novel proteins (Kinsella, 1976).

Compared to acid or alkali hydrolysis, enzymatic hydrolysis of protein, using selective proteases, provides more moderate conditions of process and few or no undesirable side reactions or products. In addition, the final hydrolysate after neutralization contains less salts and hydrolysis can be controlled by selection of specific enzyme and reaction factors to achieve required functionality (Madsen et al., 1997; Chiang et al., 1999; Darwicz et al., 2000). Enzymatic modification of proteins using selected proteases to cleave specific peptide bonds is widely used. The peptides produced have a smaller unit of amino acids and are more soluble compared to proteins. Thus, their functional properties are different: increased solubility over pH range,

ePrint@CFTRI

decreased viscosity, and significant changes in foaming, gelling and emulsifying properties. The functional properties of hydrolyzed proteins are governed to a large extent by their molecular size and their hydrophobicity (Turgeon, et al., 1992)

Proteolytic enzymes have been widely used for modification of food proteins to improve their functional properties. Enzyme modification have been used in fish, egg, wheat, cottonseed, soybean, groundnut, rapeseed and pea protein (Arzu et al., 1972; Hermansson et al., 1974; Spinelli and Koury, 1974; Arai et al., 1975; Cogan et al., 1981; Subba Rau and Srinivasan, 1988; Bhagya and Srinivasan, 1989; Kim et al., 1990; Chobert et al., 1996; Vioque et al., 1999; Vioque et al., 2000; Carolyane et al., 2002; Hrckova et al., 2002; Karamac et al., 2002; Cigic and Zelenik-Blatnic, 2004).

Rapeseed proteins were hydrolyzed in the past using different proteases such as alcalases, pronase or neutrases (Kim et al., 1992 a, Kim et al., 1992b). Enzymatic treatment of rapeseed protein with *Bacillus subtilis* protease, trypsin and pepsin were effective in solubilizing rapeseed protein while bromelain, papain and α-chymotrypsin were ineffective (Nakai et al., 1980). Alcalase is a well known non-specific endoprotease which broadly used in food research for the generation of protein hydrolysates (Adler-Nissen, 1986). Alcalase has been used for hydrolysis of rapeseed (Vioque et al., 1999; Vioque et al., 2000), soybean protein (Hrckova, 2002), Chicken egg white (Cigic and Zelenik-Blatnik, 2004) and lean meat (O'Meara and Munro, 1985).

In the present study enzyme modification has been used to improve the functional properties of heat coagulated mustard protein

ePrint@CFTRI

isolate and concentrate. The enzyme alcalase was used for partial hydrolysis of protein isolate and concentrate. The enzyme alcalase has broad specificity with some preference for terminal hydrophobic amino acids. The hydrolysates had less bitter taste (Lahl and Braun, 1994). The optimum conditions for hydrolysis were temperature 50°C, time 60 min, pH 8 (Vioque et al., 1999). The E/S ratio of 1:100 (v/w dry protein) was selected to obtain the optimum solubility of hydrolysate with improved functional properties.

Figure 44 shows the protein solubility profile of meal protein hydrolysate (DH 9.4 ± 0.1) compared to protein isolate and dehulled defatted meal at different pH values. Figure 45 shows 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. In both cases, protein hydrolysates showed a good solubility at all pH values. The solubility of protein isolate prepared from meal was in the range of 25-40% which increased to 60-75% while the protein concentrate from low hull fraction had a solubility of 20-35% which increased to 50-65% on hydrolysis.

Enzymatic hydrolysis changed the protein solubility profile from usual U shape to a fairly flat plateau (Adler-Nissen and Olsen, 1979). The flat solubility curve is of considerable advantage in many food systems as it prevents occasional precipitation problems due to otherwise unavoidable fluctuation in pH (Adler-Nissen et al., 1983).

ePrint@CFTRI

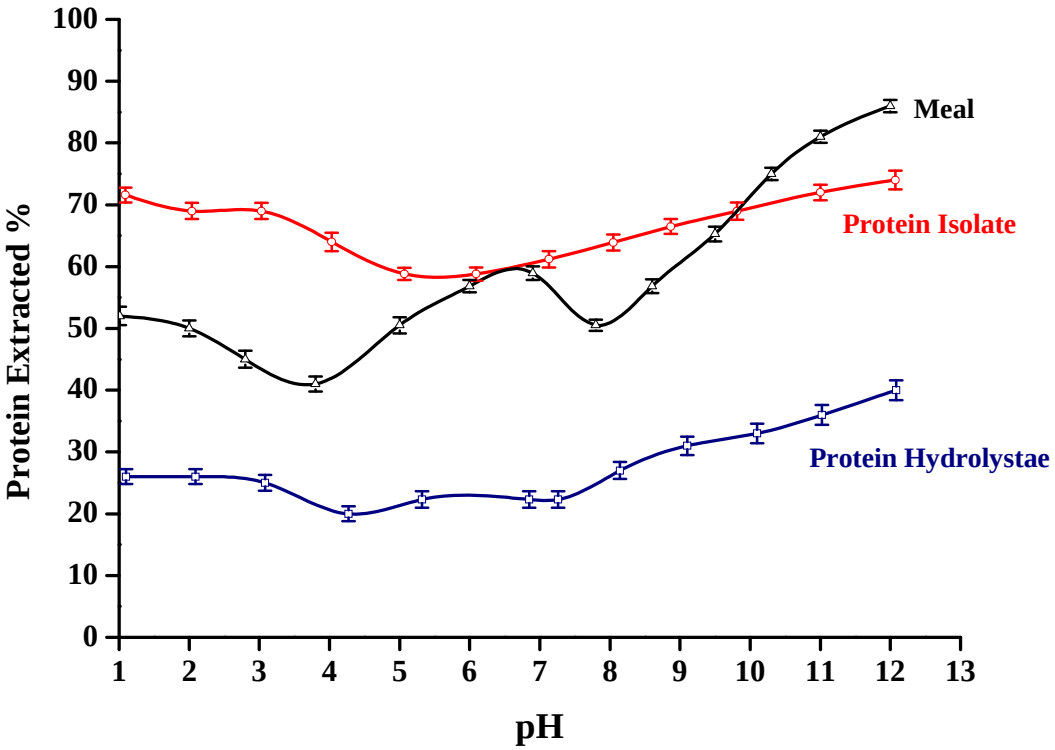


Fig 44. Protein Solubility Profile of Dehulled Defatted Meal, Protein Isolate and Hydrolysate

ePrint@CFTRI

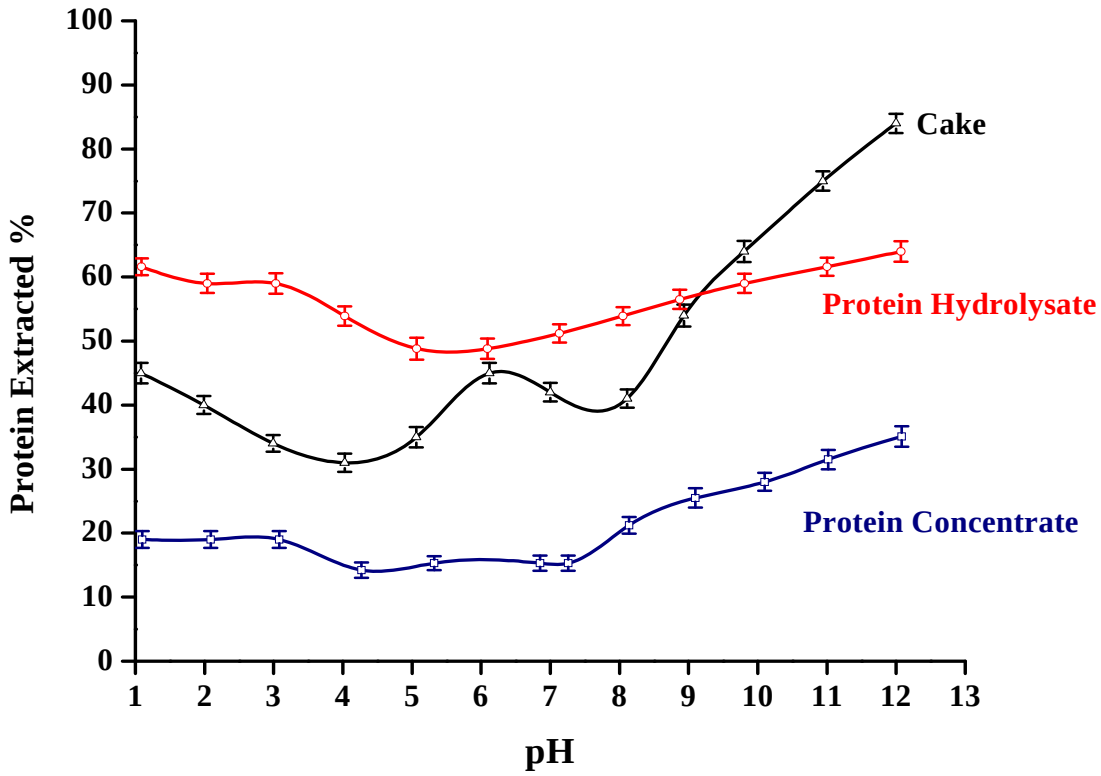


Fig 45. Protein Solubility Profile of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

ePrint@CFTRI

Increased protein solubility with enzymatic hydrolysis is well-documented (Chobert et al., 1988a; Chobert et al., 1988b; Turgeon et al., 1992; Chobert et al., 1996). This increase in solubility is due to the smaller size of peptides and higher hydrophilicity of the hydrolysates (Mahmoud, 1994). Nakai et al. (1980) have reported that treating the rapeseed, soybean and sunflower protein isolate by surfactant and protienase improve the protein dispersibility. Vioque et al. (1999) have shown that extensively hydrolyzed rapeseed protein can be obtained by the sequential use of the enzymes alcalase and flavourzyme. The protein hydrolysate had higher solubility of 100% at pH range of 2.5 to 7. The hydrolysate can be used in clinical diet or for supplementation of liquid food or high energy beverages. Limited proteolysis of rapeseed protein isolate has been shown to improve functional properties such as water absorption, oil absorption, whippability, foam capacity and foam stability, emulsion activity and emulsion stability (Vioque et al., 2000).

Limited protein hydrolysis has been shown to improve functional properties of the original material, but above a certain degree of hydrolysis (DH) these properties disappear as a consequence of the smaller peptide size. Panyam and Kirala (1996) have reported that protein hydrolysate with DH between 1 to 10% possess better functional properties than the original proteins. It has been demonstrated that limited hydrolysis improve the functionality with reduced bitterness (Puski, 1975; Zakaria and McFeeters, 1978; Adler-Nissen and Olsen, 1979; Bobalik and Taranto, 1980; Adler-Nissen, 1981).

ePrint@CFTRI

SDS-PAGE

The SDS-PAGE pattern of total protein from dehulled defatted meal, protein isolate and protein hydrolysate is given in Figure 46. The meal protein resolved into three major and eight minor bands. The molecular weights of the major bands were 34,000; 28,000; and 20,000 Da. The minor bands had the molecular weights ranged from 33,000 to 60,000 Da. On the other hand, the protein isolate showed three major bands with similar molecular weight to that of total proteins from meal. However, the protein hydrolysate did not show any bands corresponding to the above three subunits. This may be due to enzyme hydrolysis of protein which decreased the molecular weight of proteins.

Gururaj Rao and Narasinga Rao (1981) have reported that in the SDS-PAGE pattern of mustard and rapeseed proteins three major bands corresponding to molecular weight of 34,000; 27,000; and 20,000 Da. were prominent. However, the intensity of the bands corresponding to molecular weight 50,000; 14,000, and 11,000 in rapeseed was higher. This was attributed to difference in the subunit composition of the two proteins. Prakash and Narasinga Rao (1986) have reported that the 12S protein of rapeseed was separated into eight subunits with molecular weights ranged from 11,000 to 70,000 Da.

The Figure 47 shows the SDS-PAGE pattern of total protein of low hull fraction of cake, protein concentrate and hydrolysate prepared from this fraction. The proteins from low hull fraction of cake and protein concentrate prepared from it showed three major bands and there was no difference between their patterns. The patterns were similar to that of proteins from dehulled defatted meal

ePrint@CFTRI

and protein isolate prepared from it (Fig 46). However the band corresponding to higher molecular weights were not prominent as seen in the case of dehulled defatted meal. This may be due to the processing of cake by Ghani pressing which results in heat development that denatures the proteins. These results are in good agreement with low solubility of proteins in low hull fraction of cake (Fig 29, Chapter 2).

The fractions obtained from Ghani pressed cake meal were also used to study difference in the total proteins by SDS-PAGE (Fig 48). All the fractions showed similar pattern with three major bands and were comparable to those obtained for dehulled defatted meal (Fig 46). These results clearly show that the fractionation has no effect on the subunits of proteins. However, these fractions showed difference in terms of hull and protein content.

ePrint@CFTRI

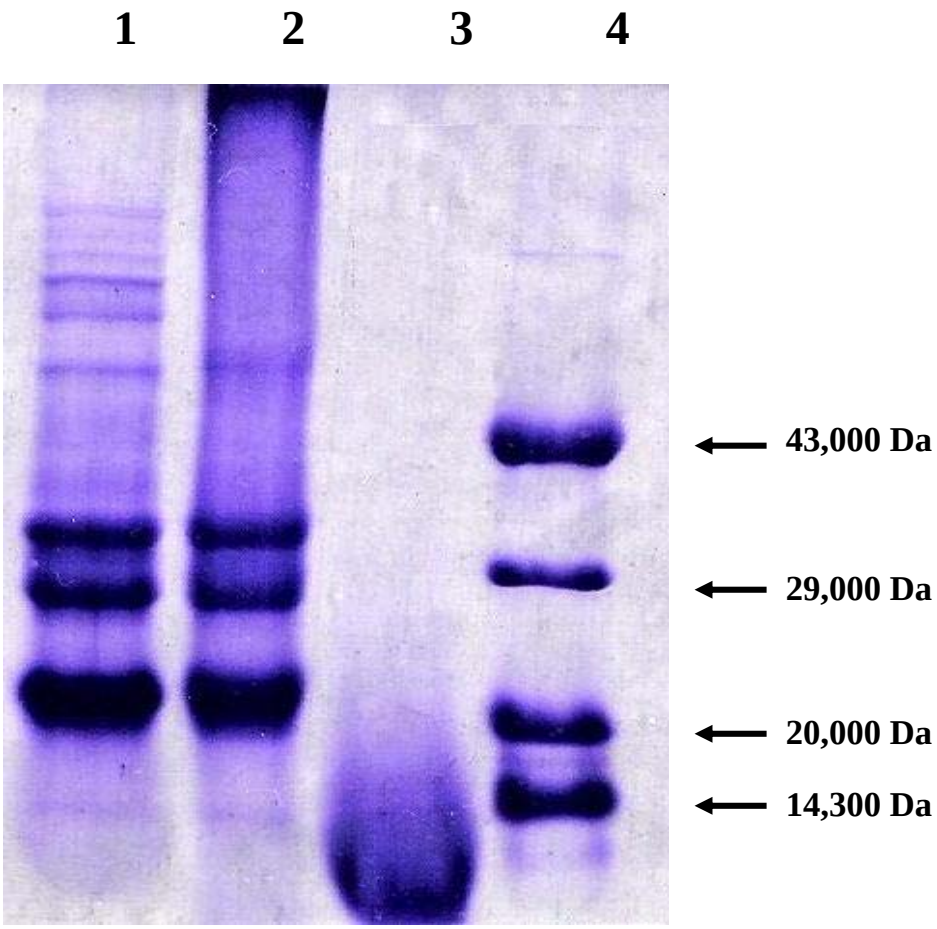


Fig 46. SDS-PAGE Pattern of Dehulled Defatted Meal (1); Protein isolate (2); Protein Hydrolysate (3); and Markers (4)

ePrint@CFTRI

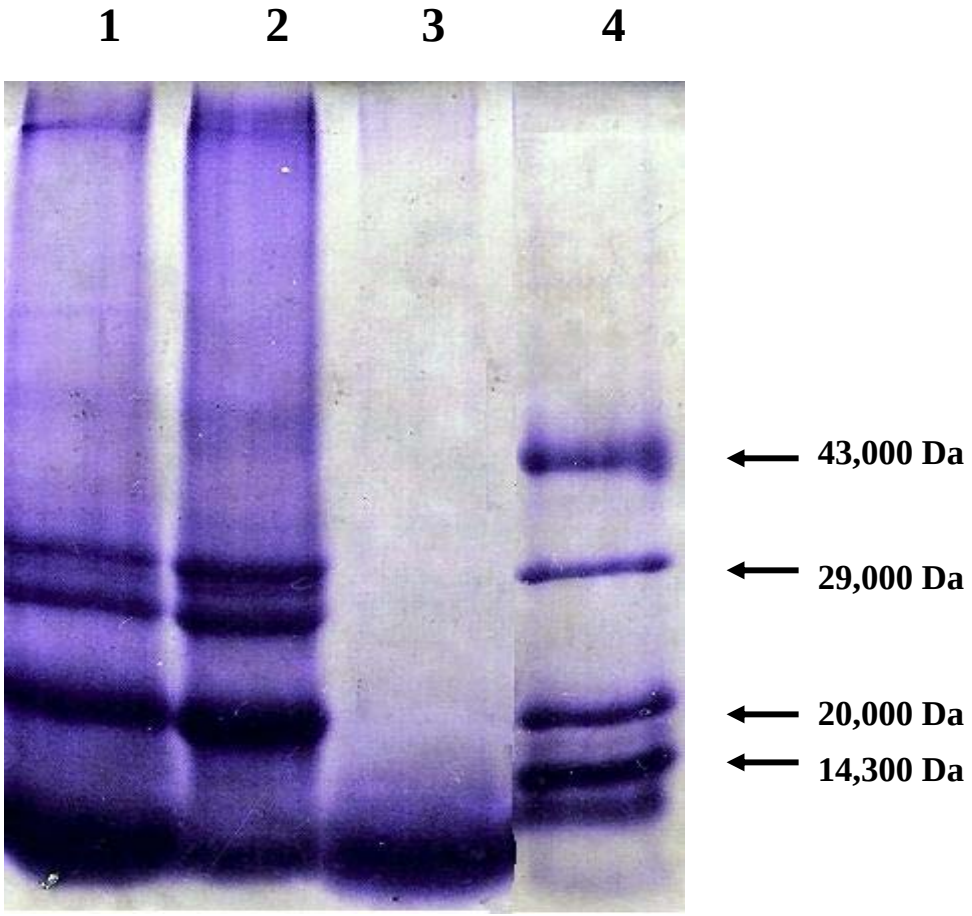


Fig 47. SDS-PAGE Pattern of Low Hull Fraction of Cake (1); Protein Concentrate (2); Protein Hydrolysate (3); and Markers (4)

ePrint@CFTRI

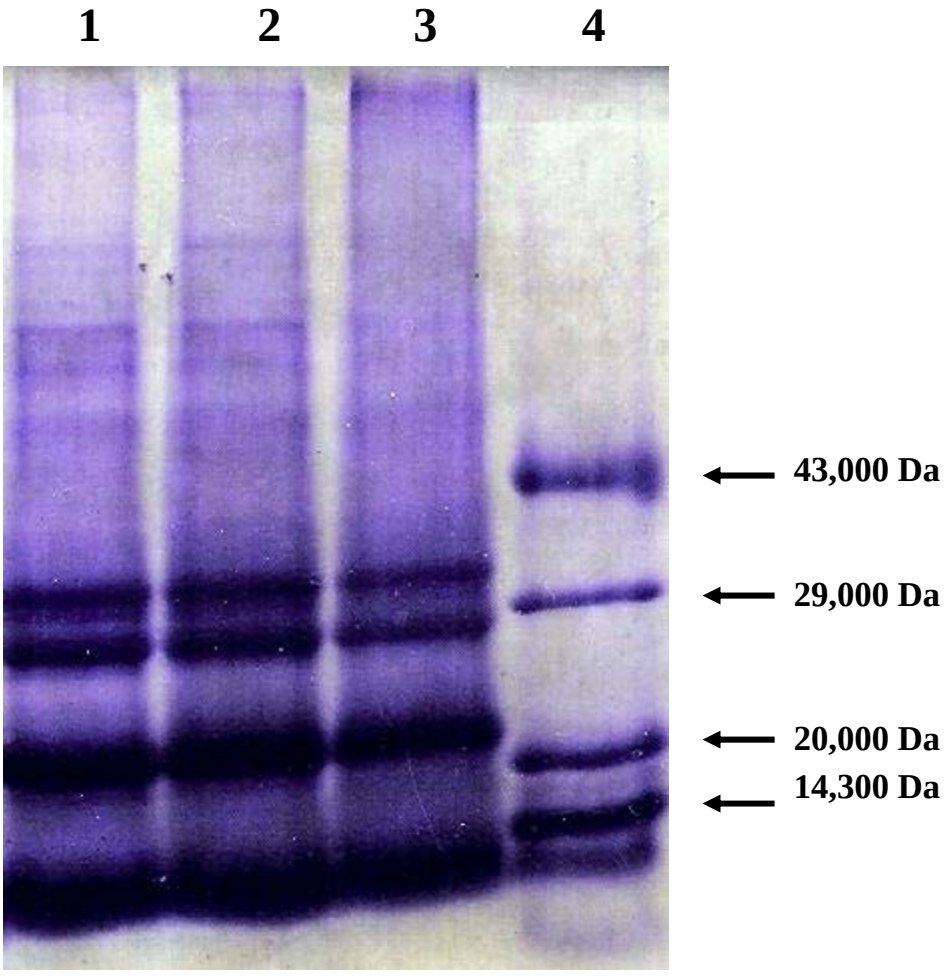


Fig 48. SDS-PAGE Pattern of Low Hull High Protein Fine Fraction (1); Low Hull High Protein Coarse Fraction (2); High Hull Fraction of Cake (3); and Markers (4)

ePrint@CFTRI

Water Absorption Capacity (WAC)

Water absorption is defined as the water absorbed by a dried protein powder after equilibration against water vapor of a known relative humidity. The ability of food ingredient to bind and structure water is an important feature that is being increasingly explored for the fabrication of novel foods (Labuza et al., 1972).

The water binding capacity of different proteins must be determined to facilitate adjustments in food formulations when interchanging protein sources. Thus, some proteins with high water binding capacities, when added to a formula, may imbibe a disproportionate amount of water and dehydrate other components in food systems or vice versa. Water binding capacity varies with protein, source, composition and the presence of carbohydrates (i.e. hydrophilic polysaccharides), lipids, pH, and salts. It may be influenced by previous processing, such as heating, alkali processing, disulfide linking, etc. (Hermansson and Akesson, 1975; Hermansson, 1975; Kinsella, 1976).

Water absorption capacity of protein isolate and protein hydrolysate prepared from dehulled defatted meal, proteins concentrate and hydrolysate prepared from low hull fraction of cake compared to the starting materials and defatted soybean meal are presented in Figure 49. The WAC of dehulled defatted mustard meal and soybean meal were 275 and 280g/100, respectively. The results showed that there was no significant difference ($P<0.05$) between WAC of defatted soybean meal and defatted mustard meal (Fig 49). On the other hand, the WAC of protein isolate was reduced to 210g/100g while the WAC of protein hydrolysate prepared from the meal increased to 260 g/100g. The decrease in WAC of protein

ePrint@CFTRI

isolate may be due to heat treatment used for coagulation of protein. The lower Water holding capacity has been reported in heat treated oilseed protein by Khalil et al. (1985). This may also be due to denaturation of protein and decreased amount of soluble protein (Fig 44).

Improved WAC as a result of enzyme hydrolysis has been reported for canola/rapeseed and groundnut (Jones and Tung, 1983; Subba Rau and Srinivasan, 1988; Vioque et al., 2000). Improvement in WAC by enzyme treated groundnut protein was attributed to increased number of polar sites exposed due to hydrolysis (Beuchat et al., 1975). Conformational changes due to heat, ionic environment and also other constituents such as carbohydrates and fiber may also influence the WAC (Narayana and Narasinga Rao, 1982).

The WAC for the low hull fraction of cake was 270 g/100g and decreased to 204 g/100g in protein concentrate. On the other hand, enzyme modification improved the WAC in the protein hydrolysate to 256g/100g. The WAC of canola/rapeseed protein products has been reported in the range of 200 and 400 g/100g (Sosulski et al., 1976; Dev and Mukherjee, 1986).

ePrint@CFTRI

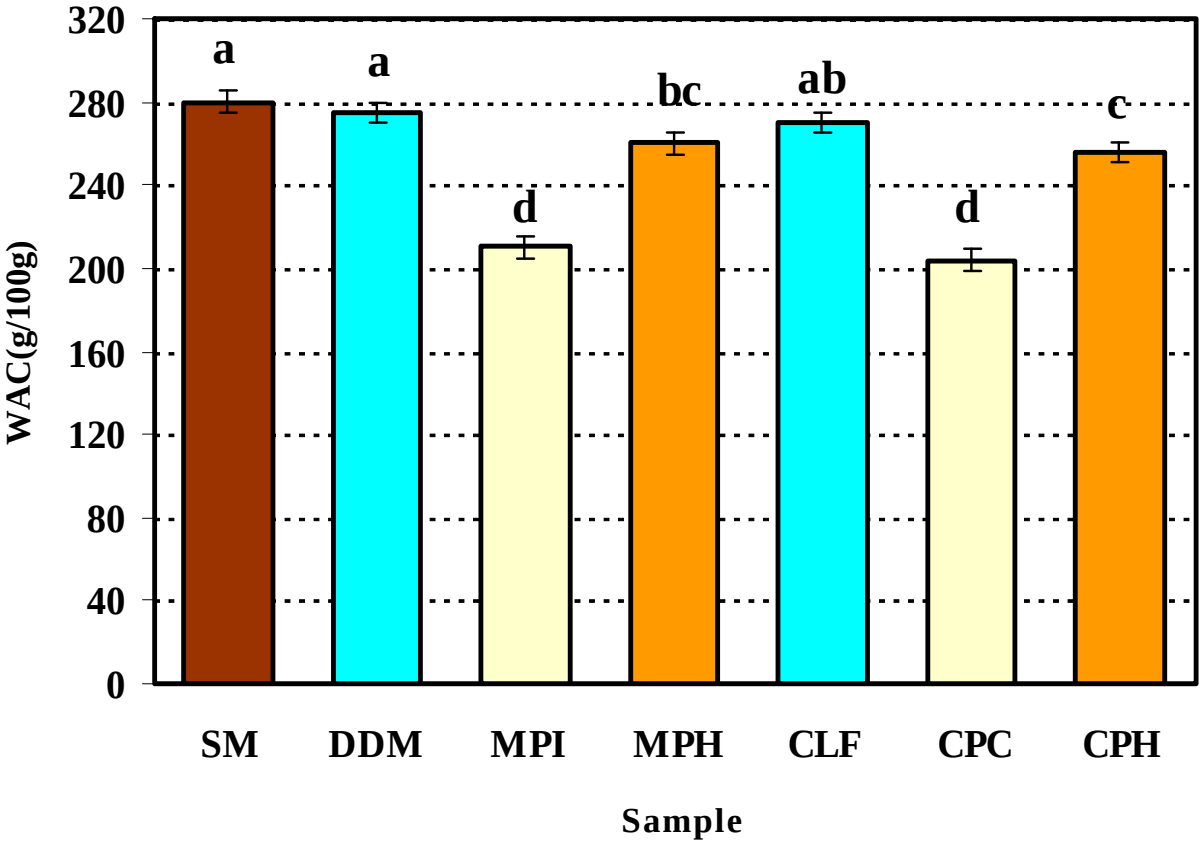


Fig 49. Water Absorption Capacity of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

Fat Absorption Capacity (FAC)

The ability of protein to bind fat is very important for such applications as meat replacers and extenders, principally because it enhances flavor retention and improves mouth fell. The FAC values are expressed as ml oil absorbed per 100g sample (Kinsella, 1976).

Fat absorption capacity of dehulled defatted mustard meal, protein isolate and hydrolysate prepared from meal, low hull fraction of cake, protein concentrate and hydrolysate prepared from this fraction are given in Figure 50. Defatted soybean meal was used for comparison. The mustard meal showed the higher FAC of 180 ml/100g sample compared to soybean meal (130 ml/100g). In protein isolate the FAC decreased to 90 ml/100g. Upon enzyme modification the FAC value improved to 130 ml/100g. However, the FAC of low hull fraction of cake was 160 ml/100g and it reduced to 90 ml/100g in protein concentrate which improved due to enzyme modification to 132 ml/100g. The results were similar to that observed for mustard meal, isolate and hydrolysate. Lin et al. (1974) have reported that heat treatment reduced functional properties including FAC of sunflower proteins.

FAC was found to have close negative correlation with the bulk density of the samples (Fig 51). The protein isolate and concentrate had high bulk density and low FAC while others had low bulk density and higher FAC. Such relation exists possibly because FAC and WAC are due to the physical entrapment of the molecules by meals or isolates (Kinsella, 1976).

ePrint@CFTRI

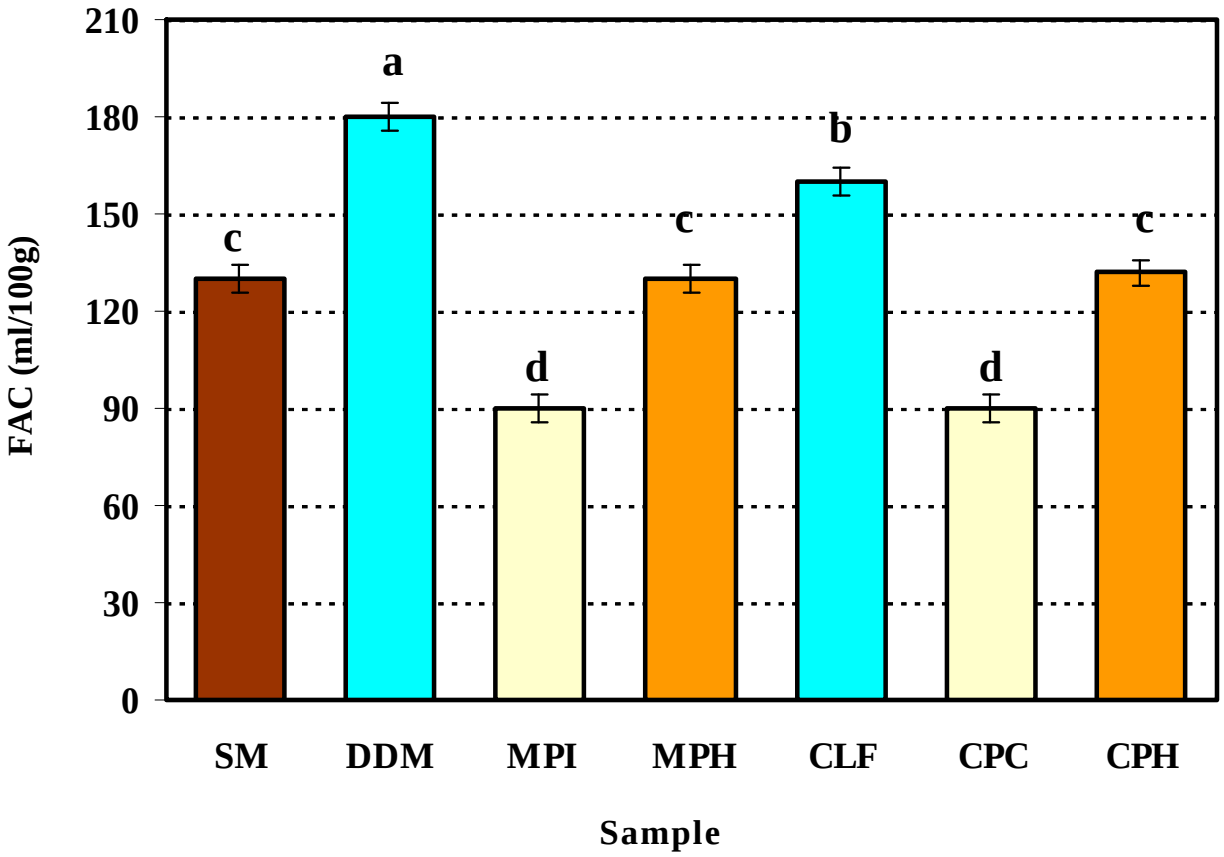


Fig 50. Fat Absorption Capacity of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

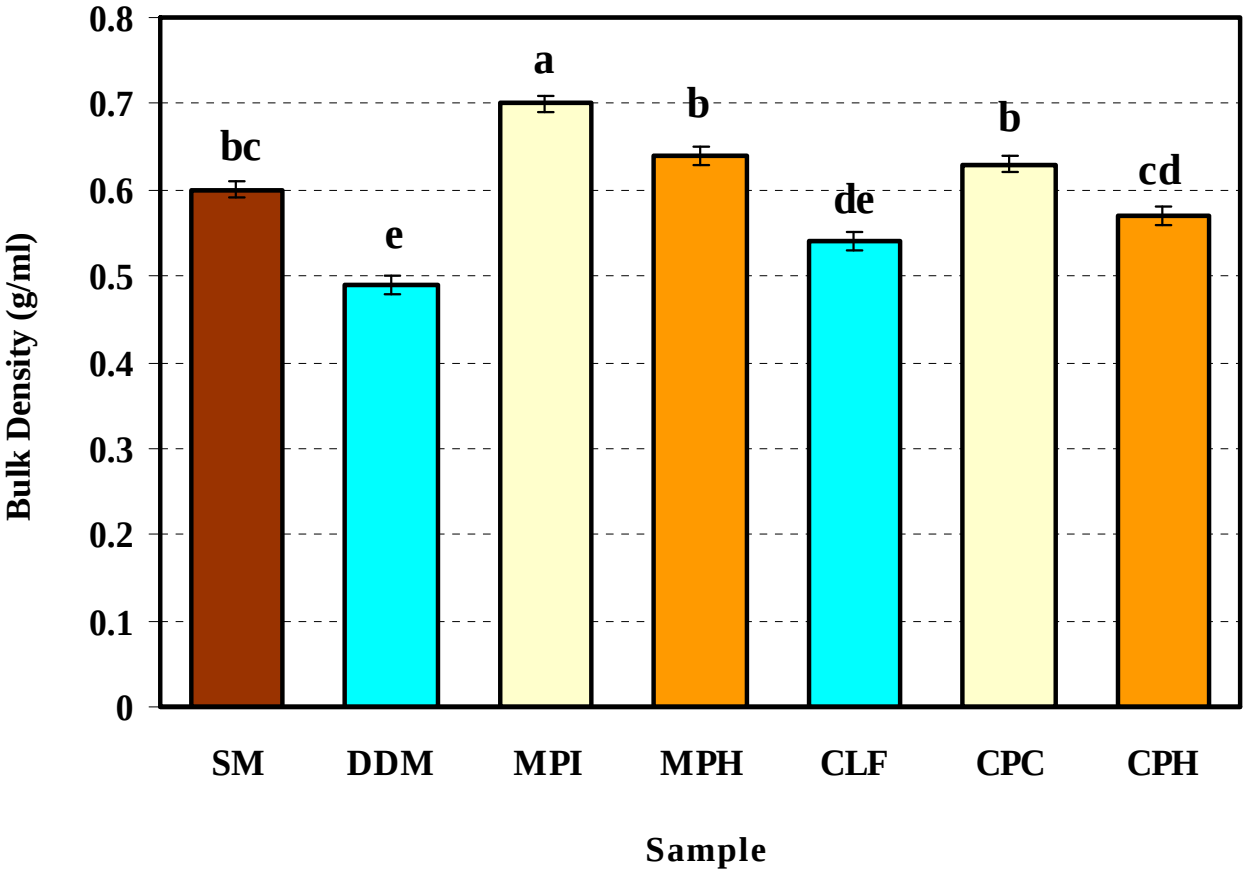


Fig 51. Bulk Density of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

Wang and Kinsella (1976) reported that alfalfa protein after extraction with acetone showed an increase in bulk density and decrease in FAC. Vioque et al. (2000) reported that rapeseed protein hydrolysate showed higher oil holding capacity than the original protein isolate. Enzymic digestion of protein exposes non-polar side chains that bind hydrocarbon moieties of oil, contributing to increased oil absorption.

Foam Capacity (FC) and Foam Stability (FS)

Foams are biphasic colloidal systems, with a continuous liquid phase and a dispersed gas bubble phase. Some food proteins are capable of forming good foams, and their capacity to form and stabilize foams depends on the type of protein, degree of denaturation, pH, temperature and whipping methods (Kinsella, 1976).

The FC and FS of mustard meal, protein isolate and hydrolysate prepared from meal, low hull fraction of cake, protein concentrate and hydrolysate prepared from low hull fraction of cake was determined at pH 7. The result were compared to defatted soybean meal and presented in Figure 52 and 53. Mustard meal had better FC and FS compared to soybean meal. The FC and FS were 110 and 100% in mustard meal compared to 85 and 77% for soybean meal. Rapeseed proteins are generally reported as having better foaming properties than soy proteins (Sosulski et al., 1976; Thompson et al., 1982a; Dev and Mukherjee, 1986).

ePrint@CFTRI

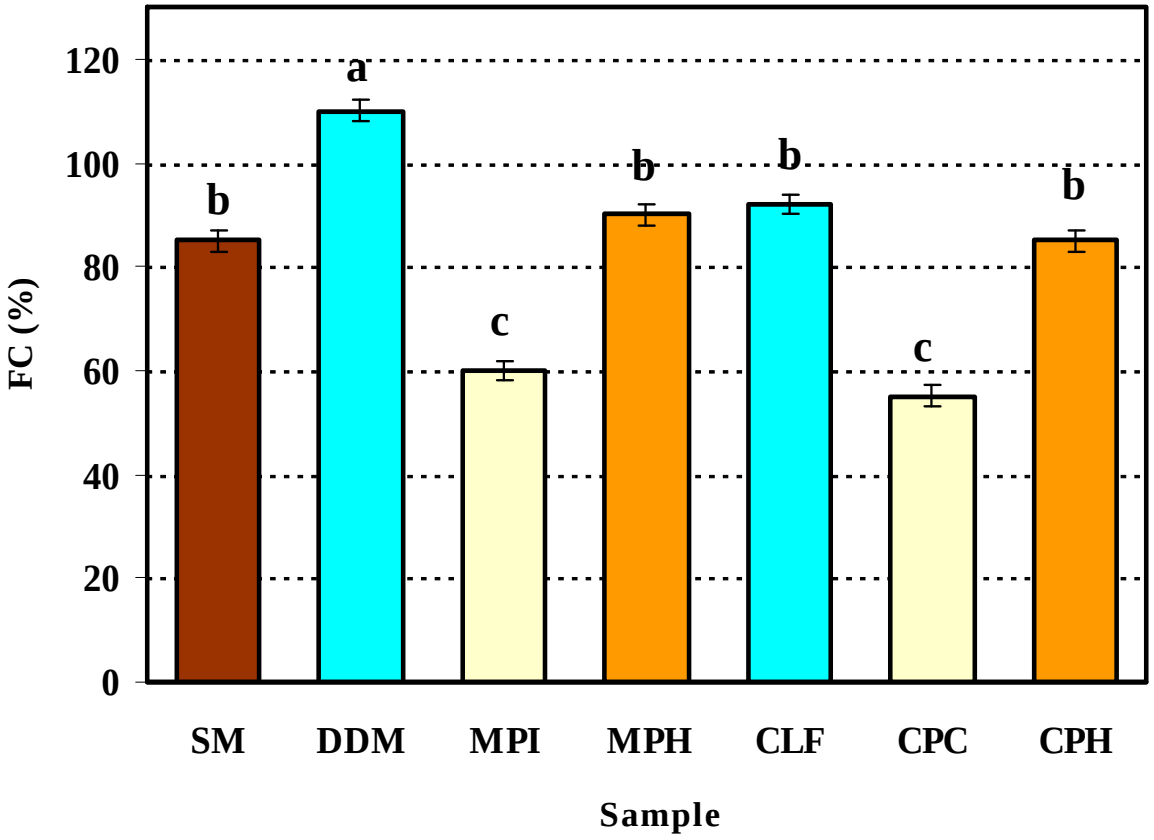


Fig 52. Foam Capacity of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

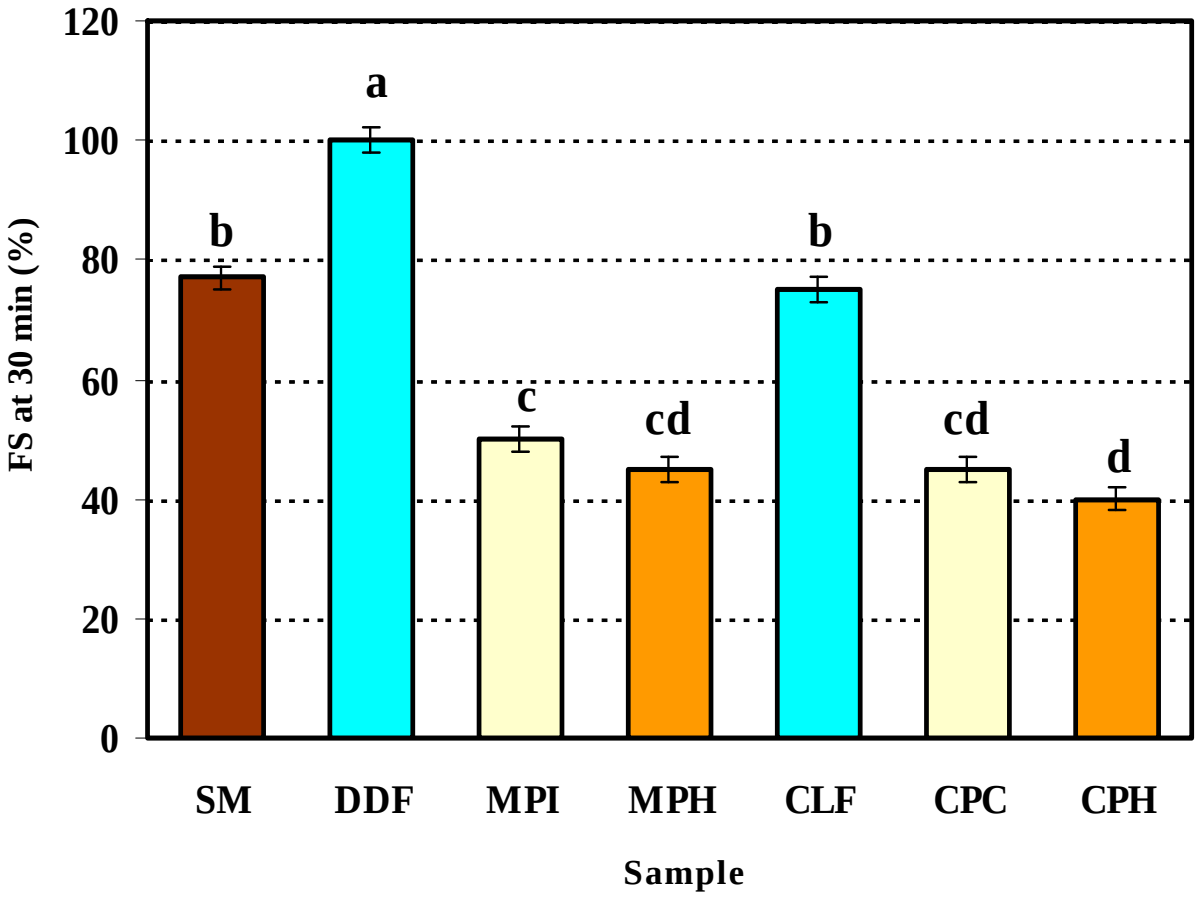


Fig 53. Foam Stability of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

The FC and FS was reduced from 110 and 100% in the meal to 60 and 50% in protein isolate, respectively. Similar reduction was observed in the case of mustard protein concentrate also. However, enzyme hydrolysis slightly improved the foam capacity of protein hydrolysates, but reduced the foam stability drastically.

It has been reported that limited proteolysis may improve foaming capacity but decreases foam stability. Rapeseed protein hydrolysate showed higher foam capacity than the original protein isolates/concentrates. Apparently, hydrolysates are capable of foaming but lack strength to maintain the foam as a result of reduction in molecular (peptide) size. The stability of foam was decreased with increased DH (Subba Rau and Srinivasan, 1988; Vioque et al., 2000).

Emulsion Capacity (EC)

The ability of protein to aid the formation and stabilization of emulsions is critical for its application in cake, butters, coffee whiteners, milk, etc. Emulsion or emulsifying capacity (EC) is usually defined as the volume of oil (ml) that can be emulsified by a gram of protein before phase inversion or collapse of emulsion occurs. There are many factors in the determination that affect the EC of protein. Some of the factors that affect the EC are equipment design, shape of container, speed of blending, rate of oil addition, temperature, pH, protein source, solubility and concentration, kind of oil used, salt (type and concentration), sugars and water content (Kinsella,1976).

The EC of mustard meal, protein isolate and hydrolysate prepared from meal, low hull fraction of cake, protein concentrate and hydrolysate prepared from low hull faction of cake was

ePrint@CFTRI

compared to soybean meal and presented in the Figure 54. The EC of mustard meal and low hull fraction of cake was significantly lower than the soybean meal. While the EC of isolate and concentrate were much lower than the original materials with a reduction of 50%. However, the enzyme hydrolysis improved the EC of both isolate and concentrate.

Dev and Mukherjee (1986) have reported that rapeseed products generally have lower emulsifying capacities than soy products, although processing treatment can alter this result. The low EC in protein isolate and concentrate may be due to heat treatment used during coagulation of protein. Heat processing markedly decreased the EC of winged bean flour (Narayana and Narasinga Rao, 1982).

Crenwelge et al. (1974) compared the emulsification capacities of protein concentrate from soy, cottonseed, bovine heamoglobin and non-fat dry milk and observed that there was a correlation between emulsifying capacity and solubility. Kinsella (1976) reported those processing factors which facilitate denaturation of soluble proteins (e.g. cyclic freezing and thawing, high temperatures, and low pH values) invariably reduce the EC and ES.

The increased EC in protein hydrolysates was perhaps due to higher solubility of enzyme treated protein and also due to exposure of hydrophilic amino acid residues as a result of proteolysis.

ePrint@CFTRI

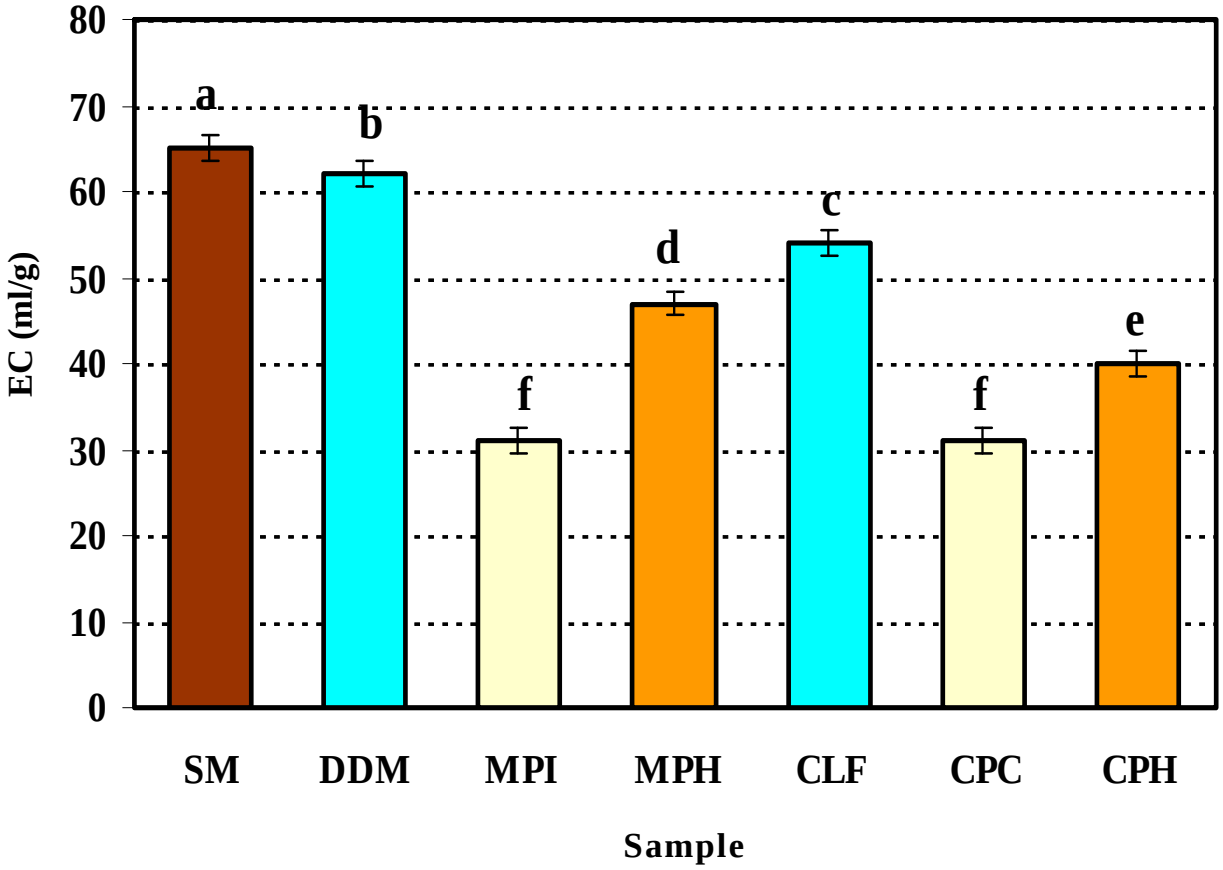


Fig 54. Emulsion Capacity of Different Samples

SM: Soybean Meal; DDM: Dehulled Defatted Meal; MPI: Meal Protein Isolate; MPH: Meal Protein Hydrolysate; CLF: Low Hull Fraction of Cake; CPC: Cake Protein Concentrate; CPH: Cake Protein Hydrolysate

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

Generally limited hydrolysis improves the emulsifying properties of proteins owing to the exposure of hydrophobic amino acid residue that may interact with the oil while the hydrophilic residues interact with water (Vioque et al., 2000). Enzymatic modification has been shown to increase the EC of groundnut protein and soy protein isolates (Bhagya and Srinivasan, 1989; Kim et al., 1990).

ePrint@CFTRI

Protein quality is dependent on the amount, distribution and availability of the essential amino acids of the protein. Various methods have been developed for assessing the protein nutritional value. The rat assay and microbiological assays have been widely used for determination of overall protein quality and availability of different amino acids. *In vitro* enzymatic assay procedures and indirect assay utilizing human subjects (Bodwell et al., 1980) are also used. Methods have also been developed to evaluate protein quality from the amino acid composition. For these evaluations, the amino acid pattern of protein source is usually "scored" against some reference pattern of essential amino acids. Generally the amino acid profiles of high quality proteins such as egg or milk proteins are used as reference pattern (Bodwell and Hopkins, 1985).

The *in vitro* methods of protein evaluation are useful in screening new protein foods because of their rapidity (Akeson and Stahman, 1964). The *in vitro* digestion by digestive enzyme like pepsin and pancreatin is sensitive method. The widely used procedure for screening protein quality are chemical score (FAO, 1968) and the essential amino acid index (Oser, 1951) based on the amino acid composition. These procedures are less time consuming and in many cases accurate. When a large number of protein samples have to be evaluated, the essential amino acid index and chemical score are very useful to evaluate the protein quality.

The protein efficiency ratio (PER) coefficient is most widely applied in evaluating the biological quality of protein, particularly in the food industry, to improve different methods of processing. The method is recommended for evaluating change in protein biological value in processed foods (AOAC, 1990). The officially approved

ePrint@CFTRI

PER assay for protein quality utilizes the rat and is not applicable for the food industry and quality control procedure, particularly because of the 28 day length of the assay. The PER assay also impractical for quality control use since it can not be used in certain low-protein or high-fat foods.

In recent years, there has been considerable interest in developing rapid and suitable methods to evaluate the protein quality of foods for humans. One of theses methods is computed protein efficiency ratio (C-PER) method of Satterlee et al. (1979) based on essential amino acid content and *in vitro* protein digestibility.

The PER assay on rats has been used as a method of choice since 1919, recommended in the U.S. and Canada for determining protein levels to be printed on food labels. However, though increased knowledge about human amino acid needs, the PER shortcomings were recognized. The PER method measures the ability of a protein to support growth in young, rapidly growing rats that have much higher essential amino acid requirements than young, rapidly growing humans. This resulted in overestimation of the quality of proteins of animal origin and underestimation of the quality of plant proteins. The most remarkable difference is in sulfur-containing amino acids, which in addition to growth, are utilized by rats in fur protein synthesis. In 1993, the U.S. Food and Drug Administration (FDA) adopted the Protein Digestibility Corrected Amino Acid Score (PDCASS) as the more accurate method for evaluating protein quality and, and this was also recommended by FAO/WHO (1991) for food labeling. The PDCASS evaluates the amino acid content of food protein and the needs of 2-5 years old children, who represent

ePrint@CFTRI

the human group with the most demanding amino acid requirements, except for infants (Sikorski, 2001).

Most proteins are susceptible to quantitive and qualitative changes during processing. The nutritional value depends on the digestibility and availability of amino acids. In general, heating improves the digestibility of protein but overheating lower the biological value of proteins. Therefore, the processing methods like heat and different treatments used for detoxification of mustard protein may affect its quality. In order to explore the potential application of mustard protein in feed and food uses, assessment of the nutritional quality was necessary.

Hence, in this chapter the nutritional quality of detoxified protein products prepared from dehulled defatted meal and low hull fraction of cake were evaluated using rapid *in vitro* assay procedures. The *in vitro* digestibility (Akeson and Stahman, 1964), available lysine (Carpenter, 1960), essential amino acid index (Oser, 1951), predicted biological value (Oser, 1959), nutritional index (Crisan and Sands, 1978) and computed protein efficiency ratio (Satterlee et al., 1979) have been calculated. The PDCASS was also calculated using FAO/WHO (1991) requirement pattern for different age groups (Table 33).

ePrint@CFTRI

Table 33. FAO/WHO Essential Amino Acid Requirement Pattern (1991) for Different Age Groups (mg/g protein)

Amino Acids	2-5 years old	10-12 years old	Adults
Threonine	34	28	9
Methionine + Cysteine	25	22	17
Valine	35	25	13
Isoleucine	28	28	13
Leucine	66	44	19
Tyrosine + Phenylalanine	63	22	19
Histidine	19	19	16
Lysine	58	44	16

ePrint@CFTRI

A. Nutritional Value of Meal, Protein Isolate and Hydrolysate

The amino acid composition of dehulled defatted meal, protein isolate and hydrolysate are presented in Table 34. It can be seen from the table that the amino acid profile of the dehulled defatted meal and protein isolate are similar except for the higher content of leucine, methionine and cysteine and lower levels of lysine in the isolate. While the amino acid composition of protein hydrolysate was similar to protein isolate indicating there was no significant change in the amino acid profile after hydrolysis.The amino acid composition of isolate compare favorably with the FAO/WHO (1991) pattern and was comparable to those reported for canola and rapeseed proteins (Klockeman, et al., 1997; Tzeng et al., 1988a). Thompson et al. (1976) reported that amino acid composition of rapeseed protein isolate was either comparable or slightly higher than the original flour.

Vioque et al. (1999) have reported that there was no apparent difference between amino acid composition of the protein isolate and protein hydrolysate, indicating that the process of enzymatic hydrolysis is a mild procedure that does not affect the amino acid profile. Similar results have been reported in the case of hydrolysates prepared from soy and casein (Cogan et al., 1981; Deeslie and Cheryan, 1988).

The *in vitro* protein digestibility of mustard meal and isolate and hydrolysates are presented in Table 35. The *in vitro* digestibility of protein isolate was 92.4% which was higher than value of 81.9% for dehulled defatted meal (Table 35).

ePrint@CFTRI

Table 34. Amino Acid Composition of Mustard Dehulled Defatted Meal, Protein Isolate and Protein Hydrolysate (g/100 g protein)

Amino Acids	Dehulled Defatted Meal	Meal Protein Isolate	Meal Protein Hydrolysate
Lysine	5.30 ± 0.20	4.90 ± 0.10	5.02 ± 0.05
Methionine	2.52 ± 0.01	2.73 ± 0.04	2.80 ± 0.10
Cysteine	2.40 ± 0.02	2.94 ± 0.08	2.88 ± 0.03
Tryptophan	1.87 ± 0.07	1.55 ± 0.04	1.65 ± 0.05
Histidine	2.79 ± 0.02	2.86 ± 0.03	2.85 ± 0.02
Isoleucine	3.71 ± 0.05	3.65 ± 0.06	3.69 ± 0.05
Leucine	7.59 ± 0.10	7.75 ± 0.05	7.85 ± 0.04
Threonine	4.38 ± 0.06	4.31 ± 0.09	4.45 ± 0.02
Valine	5.27 ± 0.07	5.17 ± 0.05	5.20 ± 0.05
Phenylalanine	4.57 ± 0.09	4.51 ± 0.04	4.65 ± 0.06
Tyrosine	2.37 ± 0.11	2.26 ± 0.05	2.40 ± 0.03
Aspartic acid	7.13 ± 0.10	6.97 ± 0.08	7.20 ± 0.05
Glutamic acid	20.67 ± 0.20	20.83 ± 0.15	20.10 ± 0.18
Serine	4.45 ± 0.05	4.49 ± 0.05	4.58 ± 0.07
Glycine	5.20 ± 0.07	5.19 ± 0.04	5.07 ± 0.08
Arginine	9.82 ± 0.05	9.97 ± 0.05	10.00 ± 0.04
Alanine	4.34 ± 0.07	4.36 ± 0.10	4.25 ± 0.05
Proline	5.62 ± 0.04	5.56 ± 0.05	5.63 ± 0.03

Mean ± SD of four determinations

ePrint@CFTRI

Table 35.Nutritional Indices of Dehulled Defatted Meal, Protein Isolate and Hydrolysate Compared to Casein

Parameters		Dehulled Defatted Meal	Meal Protein Isolate	Meal Protein Hydrolysate	Casein
In vitro Protein Digestibility (%)*		81.9 ± 0.50	92.4 ± 0.6	96.1 ± 0.10	96 ± 0.50
C-PER		2.35	2.57	2.69	2.5
Essential Amino Acid Index		89.5	90.4	91.6	85.3
Predicted Biological Value		86	87	87.5	81.3
Nutritional Index		42.5	81.4	82.5	78.3
PDCASS	2-5 years old	74	79	83.2	96
	10-12 years old	97	100	100	100
	Adults	100	100	100	100
Available lysine (%)		4.8 ± 0.10	3.95 ± 0.05	4.1 ± 0.05	ND

* Mean of ± SD of three determinations

ND: Not Detected

ePrint@CFTRI

Sarwar and McDonough (1990) have reported a true protein digestibility value of 95% for rapeseed protein isolate.

Heat treatment and isolation of protein improved the *in vitro* digestibility of proteins (Mansour et al., 1993). The values obtained were comparable to those reported for soy protein isolate and pea protein concentrate. The improved digestibility may be ascribed to heat denaturation of protein during isolation of protein and removal of anti-nutritional constituents. The denaturation could improve the digestibility because of increased accessibility of peptide bond to proteolytic enzymes.

Mansour et al. (1993) reported the *in vitro* digestibility of 78.8, 83.5 and 89.6% for rapeseed meal, protein concentrate and isolate, respectively. The improved *in vitro* digestibility of heated meal and isolated protein was attributed to the destruction of trypsin inhibitor activity or reduction of tannic acid. However Dhingra and Kapoor (1985) reported that decreased *in vitro* digestibility due to the presence of tannins which bind proteins and form indigestible complexes. Singh et al. (1991) reported that phytic acid reduced the protein digestibility by interfering with protease enzymes. Phytic acid has been linked to the inhibition of digestive enzymes such as protease, lipase, and alpha-amylases (O'Dell and De Boland, 1976; Knuckles and Betschart, 1987; Knuckles, 1988).

Thermal processing of proteins results in varying level of structural changes which are dependent on the severity of heat treatment and other environmental conditions such as pH, protein concentration and product composition. Less severe thermal processes will induce unfolding and rearrangement of three-dimensional structure

ePrint@CFTRI

and possibly protein-protein interactions (thermal denaturation).Such changes generally increase the protein digestibility and thus its nutritive value. In the case of vegetable proteins and legumes in particular, optimal thermal processing denature the proteins, thus increase the chain flexibility and accessibility while causing destruction of anti-digestive and toxic factors. Also disruption of cell walls and starch granules, increase the nutritive value (Swaisgood and Catagani, 1991). Hot lye treatment during dehulling of sesame and niger seed has been shown to improve the digestibility of protein (Bhagya and Sastry, 2003).

The higher digestibility of meal protein hydrolysate (Table 35) compared to protein isolate may be due to enzyme modification that break the protein to smaller peptide chains which are more accessible to digestive enzymes. The findings are in close agreement with the improvement of nitrogen solubility (Fig 44, Chapter 3).

The chemical score calculated based on the essential amino acid composition did not show any differences however, it suggested that the limiting amino acids in meal, isolate and hydrolysate were isoleucine, aromatic amino acids and valine as the first, second and third limiting essential amino acids, respectively (Table 36). Protein isolate prepared from canola and rapeseed meal was shown to be deficient in lysine and isoleucine (Klockeman et al., 1997; Sarwar and McDonough, 1990).

ePrint@CFTRI

Table 36. Chemical Score and Sequence of Limiting Amino Acids in Dehulled Defatted Meal, Protein Isolate, Hydrolysate and Casein

Amino acids		Dehulled Defatted Meal	Meal Protein Isolate	Meal Protein Hydrolysate	Casein
Threonine		110.2	110	110.4	81.1
Methionine + Cysteine		114.8	125.7	130.7	64.9
Valine		92.6	92.2	90.1	89.5
Isoleucine		72	71.4	71.7	91.5
Leucine		110.7	114.7	112.8	126
Tyrosine+Phenylalanine		89	88.1	89.2	118.3
Lysine		106.3	99.7	99.2	117
Protein Score		72	71.4	71.7	64.9
Sequence of limiting amino acids	First	Isoleucine	Isoleucine	Isoleucine	Methionine + Cysteine
	Second	Tyrosine + Phenylalanine	Tyrosine + Phenylalanine	Tyrosine + Phenylalanine	Threonine
	Third	Valine	Valine	Valine	Valine

*Calculated based on amino acid analysis (Table 34)

ePrint@CFTRI

The chemical score and limiting amino acids of rapeseed products varied considerably, depending on the type of process. The chemical score for raw meal, toasted meal, autoclaved meal (1h), autoclaved meal (1.5h), protein concentrate and protein isolate of mustard was 77, 43, 76, 78, 71 and 65% and the first limiting amino acid was valine, tryptophan, leucine, tryptophan, valine, isoleucine, valine, respectively (Mansour et al., 1993). The calculated nutritional indices such as essential amino acid index (EAAI) and biological value (BV) of mustard protein isolate and hydrolysate were slightly higher than the meal while the nutritional index (NI) of isolate and hydrolysate was nearly doubled, indicating the excellent quality of protein.

The NI value was found to be higher than the reported values of 25, 35, 43 and 59 for milk, pork, beef and chicken, respectively (Crisan and Sands, 1978). All other calculated nutritional characteristics were higher than those reported for other oilseed proteins (Puttaraj et al., 1994; Bhagya and Sastry, 2003).

Schwenke et al. (1990) have reported that the EAAI of 86-89 and BV of 82-87 for rapeseed flour. Sosulski and Sarwar (1973) reported EAAI value of 75 for rapeseed and 79 for turnip rapeseed meals of different varieties. EAAI of the isolated rapeseed globulin and albumin fractions were 77-80 and 74, respectively (Schwenke et al., 1990). The EAAI for rapeseed meal, toasted meal, autoclaved meal (1h), autoclaved meal (1.5h), protein concentrated and protein isolate was 80.2, 65.9, 81.4, 76.5, 75.8 and 77.9, respectively (Mansour et al., 1993).

ePrint@CFTRI

The C-PER of mustard meal was 2.35 which increased to 2.57 and 2.69 in the case of protein isolate and hydrolysate, respectively. These values are comparable with C-PER value of 2.5 for casein (Satterlee et al., 1979). Kantharaj Urs and Kowsalya (1976) have reported a PER value of 2.34 for mustard protein concentrate. The improved C-PER may be due to better digestibility of protein and removal of anti-nutritional constituents in the protein isolate (Table 35 and 29). Mansour et al. (1993) have reported that processing of rapeseed products by different methods to reduce anti-nutritional factors improved the nutritional quality of protein.

The protein quality evaluation indicated by the calculated PDCAAS values for children of different age group and adults are given in Table 35. The PDCAAS scores for the age group of 2-5 years were 74, 79 and 83.2 for meal, protein isolate and protein hydrolysate, respectively with lowest score for lysine. On the other hand, the PDCAAS score for the age group 10-12 years old were 97, 100 and 100, respectively with a lowest score for lysine. In general, the PDCAAS scores for protein isolate and hydrolysate was 100 for the age group of 10-12 years old and adults, compared to PDCASS value 97 and 100 for meal for these two age groups. The protein isolate and hydrolysate represent an excellent source of dietary protein for both the age groups. Sarwar and McDonough (1990) have reported a PDCAAS score of 83 and 93 for age group 10-12 years old for rapeseed protein isolate and concentrate, respectively. Similar PDCAAS score of 100 has been reported for canola protein isolate and comparable to those reported for high-quality protein casein, egg white, isolated soy protein and ground beef (Sarwar and McDonough, 1990; Klockeman et al., 1997).

ePrint@CFTRI

The available lysine in mustard meal was 4.8% which decreased to 3.95 and 4.1% in protein isolate and hydrolysate, respectively (Table 35). The reduction of available lysine may be due to heat treatment, interaction of phenolic with protein and millard reaction. In protein, lysine is the only essential amino acid having a free (epsilon) amino group and hence most susceptible to non-enzymatic browning (Soon Rhee and Choon Rhee, 1981). Reduction of available lysine through non-enzymatic browning and food processing has been extensively reviewed by Bender (1972), Chichester (1973), Adrian (1974), Carpenter and Booth (1973). Clandinin et al. (1959) have reported that excess heating leads to undesirable millard or browning reaction and reduced the availability of amino acids, especially lysine. Rachberger et al. (1979) have shown steaming rapeseed at 100°C for 30 min did not affect lysine availability, whereas steaming for 60 min resulted in 14% reduction and autoclaving at 123°C for 90 min decreased lysine availability by 32%. A similar trend was observed by Rayner and Fox (1976) in the case of autoclaving the rapeseed meal at 120°C for period up to 3 h.

B. Nutritional Value of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

The essential and non-essential amino acid composition of low hull fraction of cake, protein concentrate and hydrolysate are presented in Table 37. It can be seen from the table that the low hull fraction of cake contains higher amounts of lysine (4.85%) and tryptophan (1.76%). While the protein concentrate and hydrolysate contains higher amounts of leucine, isoleucine, cysteine, phenylalanine and tyrosine. However there was no significant difference between amino acid composition of protein concentrate and hydrolysate. The

ePrint@CFTRI

amino acid composition of concentrate and hydrolysate prepared by this method compared favorably with the FAO/WHO (1991) pattern (Table 33). The amino acid composition of the cake showed slight difference compared to the meal. This may be due to varietals difference in mustard seed used in preparation of cake and meal. Varietal differences in amino acid composition of rapeseed protein were found to be much greater than in Safflower and Flax (Sosulski and Sarwar, 1973).

In vitro protein digestibility of low hull fraction of cake, protein concentrate and hydrolysate are presented in Table 38. The protein concentrate showed higher the *in vitro* digestibility (88%) compared to low hull fraction of cake (80.6%). The increase in *in vitro* digestibility of protein concentrate may be due to steam injection used to precipitate protein and removal of anti-nutritional factors during processing. The protein hydrolysate had *in vitro* digestibility of 94.8% which was higher than the cake and protein concentrate. The increased i*n vitro* digestibility may be due to enzyme hydrolysis of proteins.

ePrint@CFTRI

Table 37. Amino Acid Composition of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate (g/100g protein)

Amino Acids	Low hull fraction of cake	Protein concentrate from cake	Protein Hydrolysate from cake
Histidine	3.23 ± 0.05	2.95 ± 0.05	2.88 ± 0.03
Isoleucine	3.29 ± 0.03	3.60 ± 0.08	3.65 ± 0.05
Leucine	7.02 ± 0.03	7.78 ± 0.02	7.81 ± 0.04
Lysine	4.85 ± 0.05	4.25 ± 0.05	4.22 ± 0.03
Methionine	2.08 ± 0.07	2.00 ± 0.05	2.05 ± 0.05
Cysteine	2.10 ± 0.10	2.25 ± 0.02	2.31 ± 0.03
Threonine	3.67 ± 0.06	3.91 ± 0.04	3.79 ± 0.06
Tryptophan	1.76 ± 0.02	1.45 ± 0.03	1.48 ± 0.04
Valine	5.03 ± 0.05	5.16 ± 0.04	5.24 ± 0.06
Phenylalanine	4.67 ± 0.03	5.15 ± 0.05	5.35 ± 0.05
Tyrosine	2.87 ± 0.07	3.18 ± 0.02	3.31 ± 0.04
Aspartic acid	6.83 ± 0.04	7.25 ± 0.03	7.38 ±0.02
Glutamic acid	22.08 ± 0.15	20.69 ± 0.1	20.40 ± 0.12
Serine	4.78 ± 0.03	4.63 ± 0.07	4.48 ± 0.07
Glycine	5.42 ± 0.03	5.05 ± 0.05	4.82 ± 0.04
Arginine	8.39 ± 0.05	9.94 ± 0.06	10.05 ± 0.05
Alanine	5.60 ± 0.05	5.22 ± 0.03	5.20 ± 0.05
Proline	6.33 ± 0.07	5.54 ± 0.06	5.58 ± 0.06

Mean ± SD of four determinations

ePrint@CFTRI

Table 38.Nutritional Indices of Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

Parameters		Low Hull Fraction of Cake	Protein Concentrate from Cake	Protein Hydrolysate from Cake
In vitro Protein Digestibility (%)*		80.6 ± 0.5	88 ± 0.3	94.8 ± 0.2
C-PER		2.1	2.26	2.46
Essential Amino Acid Index		80.9	85.5	85.7
Predicted Biological Value		76.5	81.5	81.7
Nutritional Index		33.2	68.4	65.1
PDCASS	2-5 years old	68.8	64.3	69.2
	10-12 years old	90	85.4	91
	Adults	100	100	100
Available lysine (%)		4.45 ± 0.05	3.60 ± 0.1	3.75 ± 0.05

* Mean of ± SD of three determinations

ePrint@CFTRI

The trends of results were similar to those observed for dehulled defatted meal, protein isolate and hydrolysate prepared from meal. The C-PER of low hull fraction of cake was 2.1 which was lower than the C-PER value of 2.26 and 2.46 for protein concentrate and protein hydrolysate, respectively. The C-PER of cake protein hydrolysate (2.46) was comparable to casein (2.5). These results are in good agreement with reported value for mustard protein concentrate by Kantharaj Urs and Kowsalya (1976). The EAAI of protein concentrate and hydrolysate were 85.5 and 85.7, respectively and it was higher than the value of 80.9 for the low hull fraction of cake.

The biological value for low hull fraction of cake was 76.5 which was higher than the value of 81.5 and 81.7 for protein concentrate and hydrolysate, respectively. However the nutritional index of protein concentrate and hydrolysate were nearly double compared to cake, indicating the excellent quality of proteins.

The protein quality evaluation indicated by the calculated PDCAAS values for children of different age groups and adults are given in Table 38. The PDCAAS scores for the age group of 2-5 years were 68.8, 64.3 and 69.2 for cake, protein concentrate and hydrolysate, respectively with lowest score for lysine. On the other hand, the PDCAAS scores for protein concentrate and hydrolysate was 85.4 and 91, respectively for the age group of 10-12 year old compared to 90 for low hull fraction of cake. The PDCAAS values for adults were 100 for all the samples. The results clearly indicate that mustard proteins are excellent source of dietary protein for adults. The chemical score calculated based on the essential amino acids compositions are presented in Table 39.

ePrint@CFTRI

Table 39. Chemical Score and Sequence of Limiting Amino Acids in Low Hull Fraction of Cake, Protein Concentrate and Hydrolysate

Amino acids		Low Hull Fraction Of Cake	Protein Concentrate from Cake	Protein Hydrolysate from Cake
Threonine		98.9	101.5	97.2
Methionine + Cysteine		104.4	102.4	103.7
Valine		94.7	93.6	93.9
Isoleucine		68.5	72.2	72.4
Leucine		109.6	117.1	116
Tyrosine + Phenylalanine		103.6	110.3	113.3
Lysine		104.1	88	86.3
Protein Score		68.5	72.3	72.4
Sequence of limiting amino acids	First	Isoleucine	Isoleucine	Isoleucine
	Second	Valine	Lysine	Lysine
	Third	Threonine	Valine	Valine

*Calculated based on amino acid analysis (Table 37)

ePrint@CFTRI

The chemical score of protein concentrate and hydrolysate were 72.3 and 72.4, respectively and were higher than the value for the low hull fraction of cake. The results showed that the limiting amino acids in protein concentrate and hydrolysate were isoleucine, lysine and valine as the first, second and third limiting essential amino acids, respectively. In low hull fraction of cake isoleucine, valine and theronine were the first, second and third limiting essential amino acids, respectively.

Summarizing the results, it can be concluded that the amino acid composition of the meal, protein isolate and hydrolysate showed variations. The meal protein products were limited in isoleucine followed by aromatic amino acids and valine. All the nutritional characteristics of defatted meal were higher than the low hull fraction of cake and its protein products. The comparison between the nutritional quality of mustard protein products and casein as reference protein suggested that mustard protein isolate and hydrolysate are better source of protein.

ePrint@CFTRI

Protein deficiency in the diet particularly of young children and the vulnerable groups is the major nutritional problem in developing countries. There is an urgent need to augment food protein supplies by tapping all available sources. Oilseeds represent an intermediate and major potential for increasing the protein supplies. Attention was focused on the large availability of oilseeds in India as an important source of protein for food. With the increasing concern for improving protein quality and increasing protein content of many existing foods, and with rising prices of conventional protein-containing foods, there is interest in relatively low cost, high protein products which might be used to simulate existing foods, as additives to existing foods to produce acceptable food products at lower cost or to formulate entirely new types of foods.

The Food industry is seeking less expensive protein for use in the manufacture of modern convenience foods. Proteins, as isolates or concentrates, are necessary ingredients in many food processes, where they perform specific function. In development of new proteins, nutritional value has been a primary criterion. While this is an important characteristic, there are several other criteria which a protein intended for food use should fulfill including functional properties. While nutritionally balanced foods can be formulated from a variety of raw materials, the acceptability of many such formulations, from the point of view of appearance, flavor, odor and texture is frequently disappointing (Kinsella et al., 1985).

When replacing or substituting for traditional proteins, new proteins should maintain or improve the quality and acceptability of food products in which they are used. New proteins should have satisfactory intrinsic properties (i.e. nutritional) and acceptable flavor,

ePrint@CFTRI

color and texture. Nutritional value is of little consequence if the protein or food in which it is incorporated is not acceptable for eating. In developing new products certain criteria should be met. The product must be palatable, low cost, easy to prepare, high in nutritional value and able to be stored for reasonable periods of time under many kinds of climatic conditions.

Pasta products such as macaroni, spaghetti, vermicelli and noodles are manufactured from semolina and flour produced from durum wheat. Pasta products are becoming popular in current lifestyle because they are healthy, tasty and convenient for transportation and preparation (Cubadda, 1994). In recent years pasta has become more popular due to its nutritional properties, being regarded as a product with low glycemic index (Jenkins et al., 1988, Wolver, 1990; Bjork et al., 2000). Nutritionists consider pasta to be highly digestible and also provide significant quantities of complex carbohydrates, protein, B-vitamins, iron and low in sodium and total fat (Douglass and Matthews, 1982). These products are prepared using durum wheat. The protein content of durum wheat is around 11-15% but deficient in essential amino acids, lysine and methionine. In order to supplement these limiting amino acids, oilseed proteins, which are rich source of these essential amino acids, could be blended with wheat flour to enrich the product. Protein fortification has been used as a convenient method of increasing the nutritional value of pasta, especially for use in developing countries (Bahnassey and Khan, 1986).

Pasta products have been fortified with non fat dry milk; whey protein; milk protein; cottonseed; yeast protein concentrate; fish protein concentrate; egg albumin; soybean flour, grits, protein concentrate and

ePrint@CFTRI

isolate; powdered walnut hulls; bean protein concentrate; navy and pinto beans protein isolates; pea flour and protein concentrate; corn gluten meal; legume flour or protein concentrate (navy, pinto and lentil); Buckwheat; amaranth; lupine; fababean flour and protein concentrate and rapeseed meal (Paulsen, 1961; Glabe et al., 1967; Kwee et al, 1969; Matsuo et al., 1972; Durr, 1973;Banasik, 1975; McCormick, 1975; Siegel et al., 1975; Schoppet et al., 1976; Haber et al., 1978; Hanna et al., 1978; Lorenz et al., 1979; Morad et al., 1980; Nielsen et al., 1980; Beslagic, 1981; Seyam et al., 1983; Bahnassey and khan, 1986; Bahnassey et al., 1986; Leonardo et al., 1989; Rayas-Duarte et al., 1996; Wu et al., 2001).

Brassica protein products have been used to supplement the bakery products, meat products, instant foods and spices (Ohlson and Anjou, 1979; Ooraikal et al., 1980; Mieth et al., 1983b). In this chapter, the detoxified mustard protein isolate was used at different levels of 0, 2.5, 5, 10 and 15% to enrich the spaghetti formulation. The effects of supplementation on dough characteristics were studied.

The farinograms of spaghetti dough with different level of supplementation of semolina with mustard protein isolate (MPI) are presented in Figures 55 to 59. Three terms were used to describe the farinograms: (1) the dough development time (DDT) is the time from zero to the point of maximum consistency of dough immediately before the first indication of weakening; (2) the maximum consistency (MC) is the height in farinograph unit (FU) at the centre of the curve at the point of maximum consistency; (3) Mixing tolerance index (MTI) is the difference in farinograph unit (FU) between the top of the curve at the peak (maximum consistency) and the top of the curve measured 4 min after the peak is reached (Irvine et al.; 1961).

ePrint@CFTRI

The rheological properties of spaghetti dough with different level of supplementation of mustard protein isolate are shown in Table 40. The results showed that the maximum consistency (MC) of spaghetti dough increased from 351 FU for control to 371 and 386 FU for 2.5 and 5% supplementation, respectively. However, the supplementation of semolina with 10% and above decreased the MC of dough drastically. The increased MC of 2.5 and 5% supplementation may be due to increased amount of protein that helps to form stronger gluten network. However, reduction of MC with higher levels of supplementation could be attributed to diluting effect of mustard protein isolate on gluten.

The dough development time (DDT) increased with increasing the level of supplementation from 3.5 min for control to 15.2 for 15% supplementation in spaghetti dough. The increase in DDT may be due to increased amount of non-gluten protein that compete with gluten for water and therefore increase the time for development of gluten network. Spaghetti dough were prepared with fixed amount of water for all samples and therefore higher levels of supplementation increases the non-gluten content of dough and therefore the competition between gluten and non-gluten for available water increase.

ePrint@CFTRI

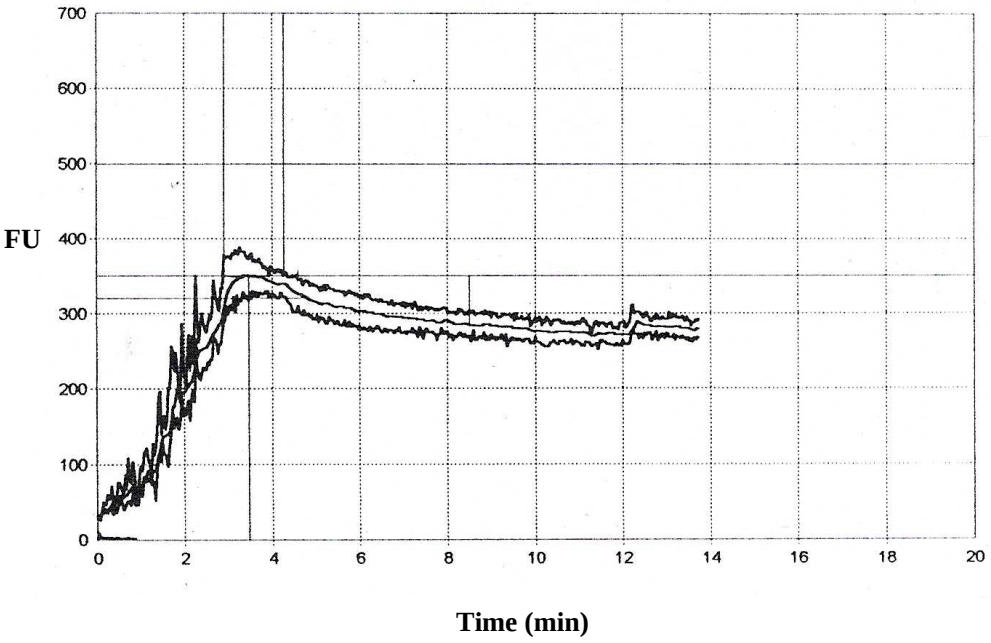


Fig 55. The Farinogram of Control Spaghetti Dough

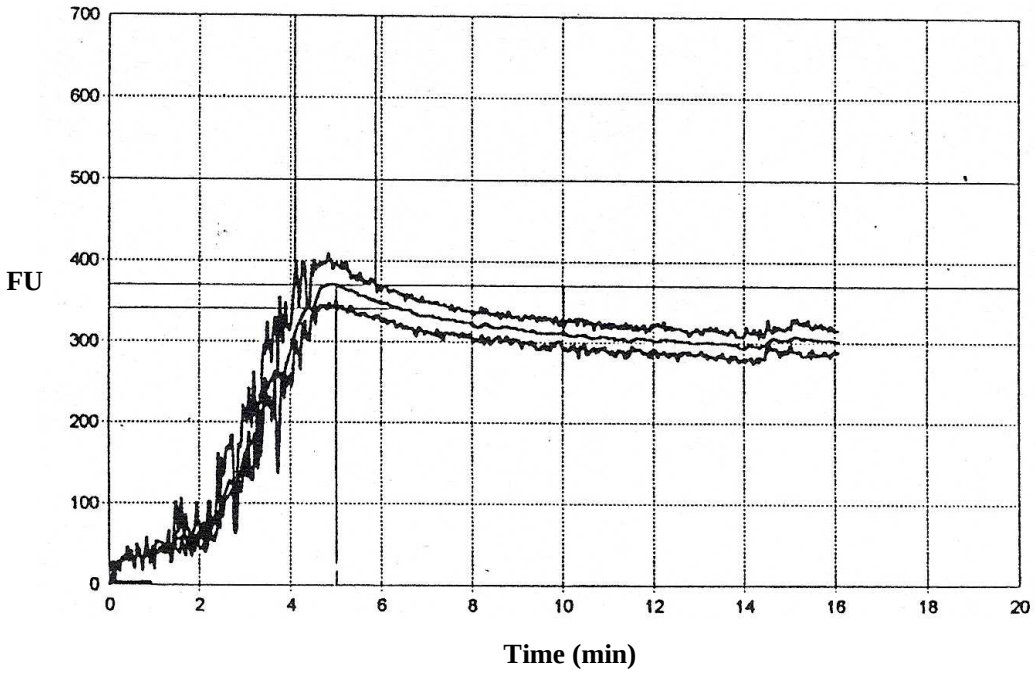


Fig 56. The Farinogram of Spaghetti Dough with 2.5% Supplementation

ePrint@CFTRI

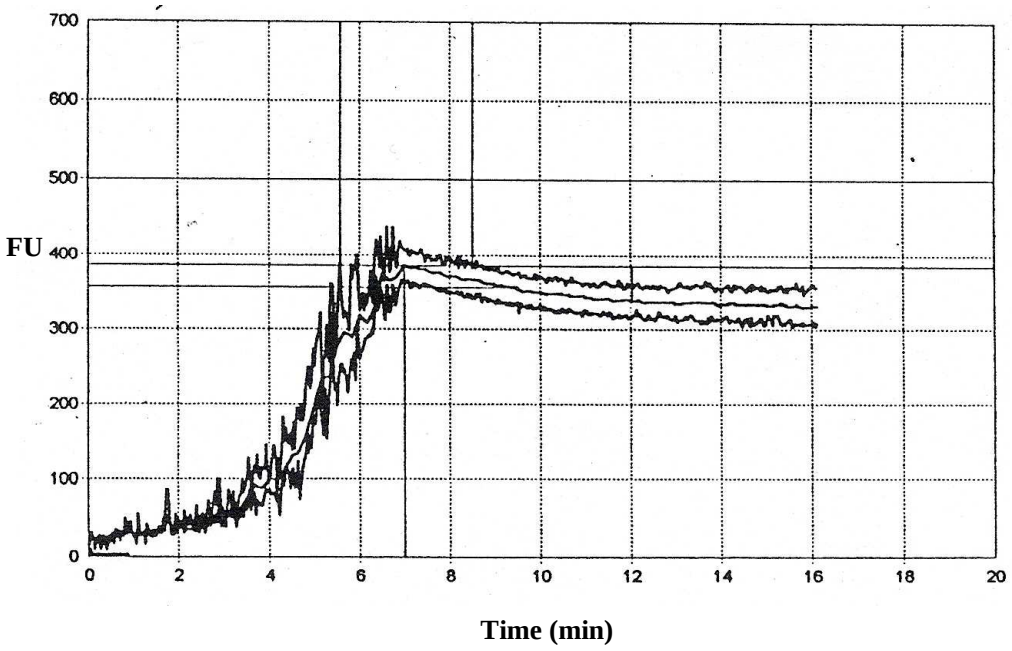


Fig 57. The Farinogram of Spaghetti Dough with 5% Supplementation

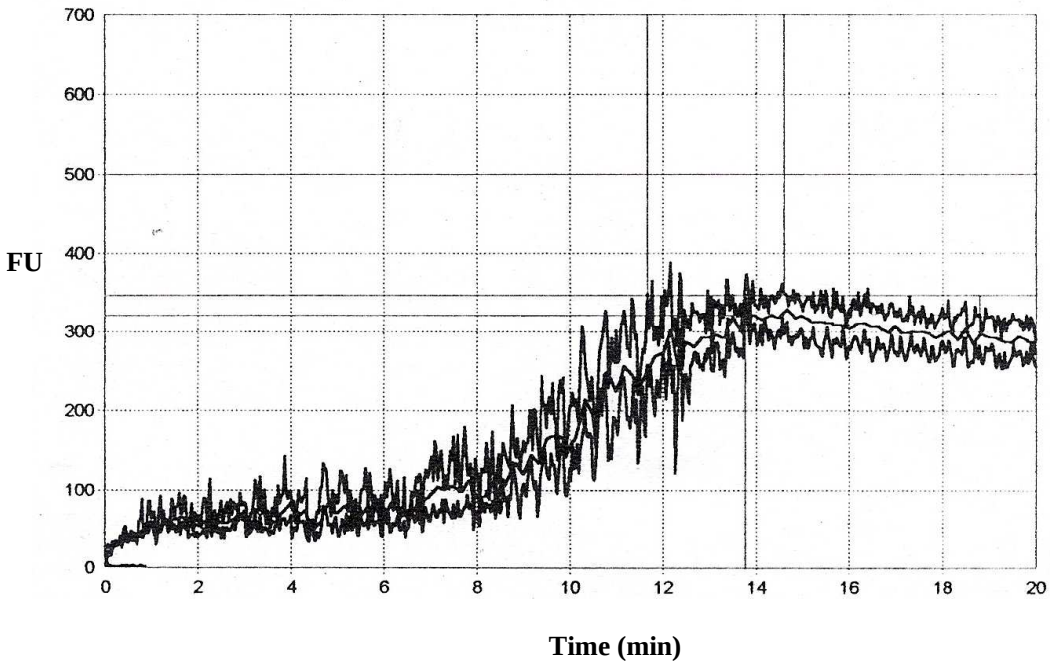


Fig 58. The Farinogram of Spaghetti Dough with 10% Supplementation

ePrint@CFTRI

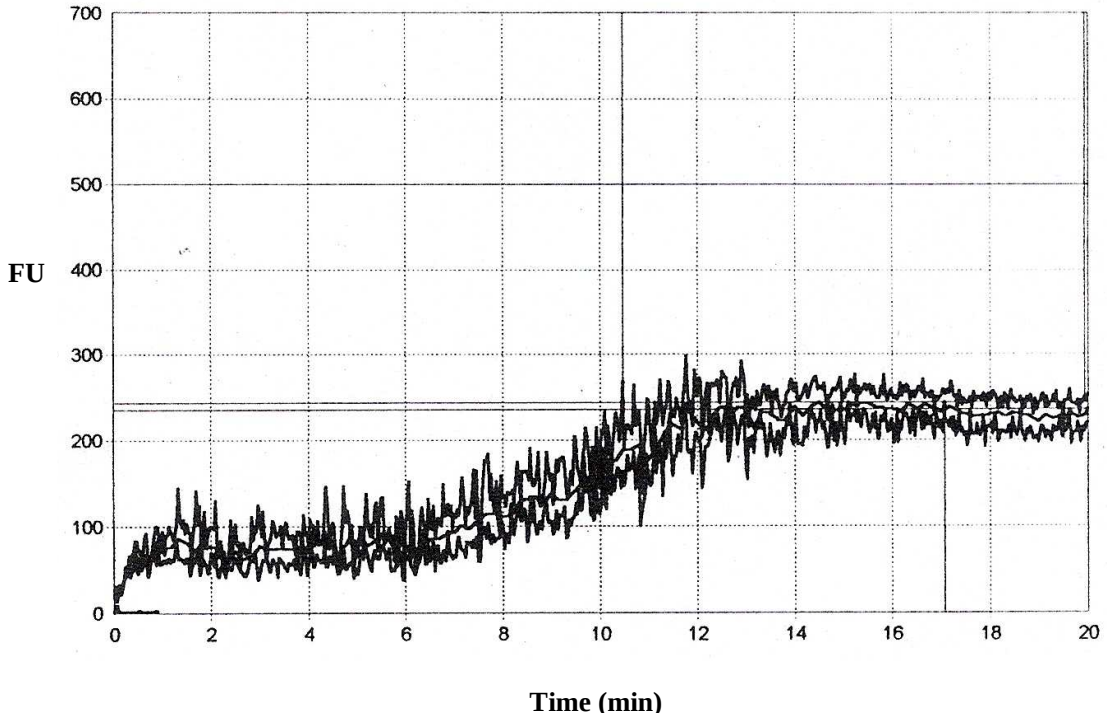


Fig 59. The Farinogram of Spaghetti Dough with 15% Supplementation

ePrint@CFTRI

Table 40. Rheological Properties of Spaghetti Dough with Different Levels of Supplementation

Supplementation Levels (%)	Dough Development Time (DDT) (min)	Maximum Consistency (MC) (min)	Mixing Tolerance Index (MTI) (FU)
0	3.5	351	59
2.5	5	371	53
5	7	386	38
10	13.8	346	23
15	15.2	243	12

ePrint@CFTRI

Irvine (1971) has reported that the ability to form a gluten matrix is unique to wheat flour and semolina and is believed to be the main factor in forming the internal spaghetti network that holds the pasta together. The addition of non-gluten flours diluted the gluten strength and interrupted and weakened the overall structure of the spaghetti (Rayas-Duarte et al., 1996).

The mixing tolerance index (MTI) decreased with increase in supplementation level of mustard protein in spaghetti dough (Table 40). Irvine et al. (1961) have reported that as the amount of protein (gluten) increases, dough development time (DDT) decreases, with increase in maximum consistency (MC) and mixing tolerance index (MTI).

However, increasing the non-gluten protein may be weakens the gluten network which increase the dough development time (DDT) and reduce the mixing tolerance index (MTI). Haber et al. (1978) have reported that the addition of the high protein soy or cotton seed meal products at 5-20% level decreased the MTI value of semolina dough.

The results indicated that supplementation of protein isolate up to 10% did not show any deteriorative effects on dough characteristics. However, higher levels resulted in negative effect on spaghetti dough characteristics. Dough prepared with 15% supplementation did not show any good handling and mixing properties for extrusion. The low consistency of this dough (Table 40) due to high amount of non gluten protein weakens the gluten network and makes the dough unsuitable for spaghetti making. Matsuo and Irvine (1970) have reported that the structure of dough can be envisaged as starch and other minor

ePrint@CFTRI

component enveloped by a three-dimensional network of gluten. The sheeting characteristics and the strength of the membrane would play a significant role in the rheology of the system and influence the behavior during processing, the rigidity of the starch during and after drying, and the properties upon cooking.

Therefore, based on the above results the supplementation levels of 2.5, 5 and 10% were used for preparation of spaghetti. The Figures 60 to 63 show the spaghetti prepared by supplementation of semolina with different level of mustard protein isolate. All the enriched spaghetti samples were evaluated for color characteristics, scanning electron microscopy, chemical composition, cooking characteristics, sensory evaluation and nutritional value.

ePrint@CFTRI

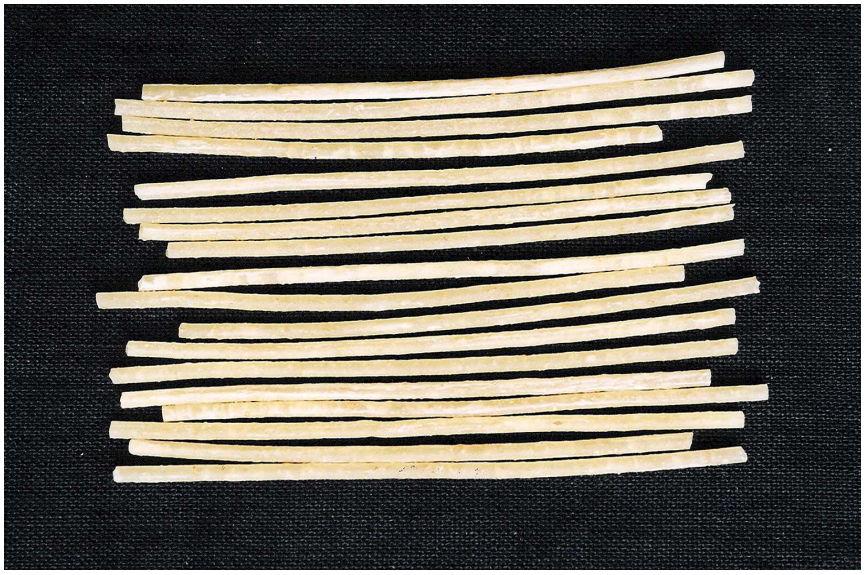


Fig 60. Control Spaghetti

Fig 61. Spaghetti with 2.5% Supplementation

ePrint@CFTRI

Fig 62. Spaghetti with 5% Supplementation

Fig 63. Spaghetti with 10% Supplementation

ePrint@CFTRI

Color Characteristics

The color characteristics of enriched and control spaghetti samples are presented in Figures 64 to 66. The *L* values (lightness) of semolina and control spaghetti were higher than enriched spaghetti samples. The *L* value decreased with the increase in supplementation level (Fig 64). This may be due to lower *L* value of mustard protein isolate (Chapter 3, Fig 32) compared to semolina. It may also be due to the effect of extrusion and drying on color characteristics of spaghetti.

The *a* values (redness) of spaghetti with different enrichment levels were higher than control and semolina. The redness increased with increased level of supplementation (Fig 65). The increased *a* values may be due to higher *a* value of mustard protein isolate (Chapter 3**,** Fig 34) compared to semolina. Changes in *a* values may also be due to the effect of extrusion and drying.

The *b* values (yellowness) of spaghetti with different levels of enrichment were higher than control or semolina, which increased with increase in enrichment levels (Fig 66). This may be due to higher *b* value of mustard protein isolate (Chapter 3, Fig 33) compared to semolina. However, compared to semolina the *b* value was slightly increased in control spaghetti. This could be attributed to processing (extrusion and drying) effect.

Wu et al. (2001) have reported that semolina color became darker (lower *L* value) and more yellow (higher *b* value) as water/ethanol washed corn gluten meal was substituted for semolina or farina.

ePrint@CFTRI

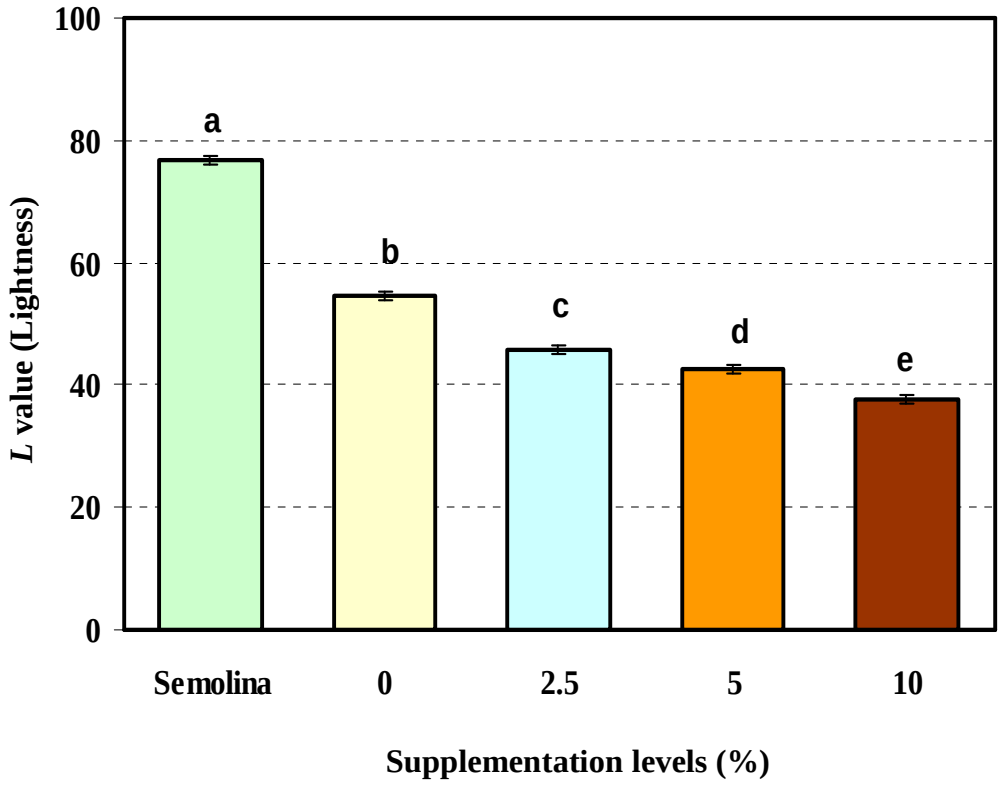


Fig 64. *L* Values of Spaghetti with Different Levels of Supplementation Compared to Semolina

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

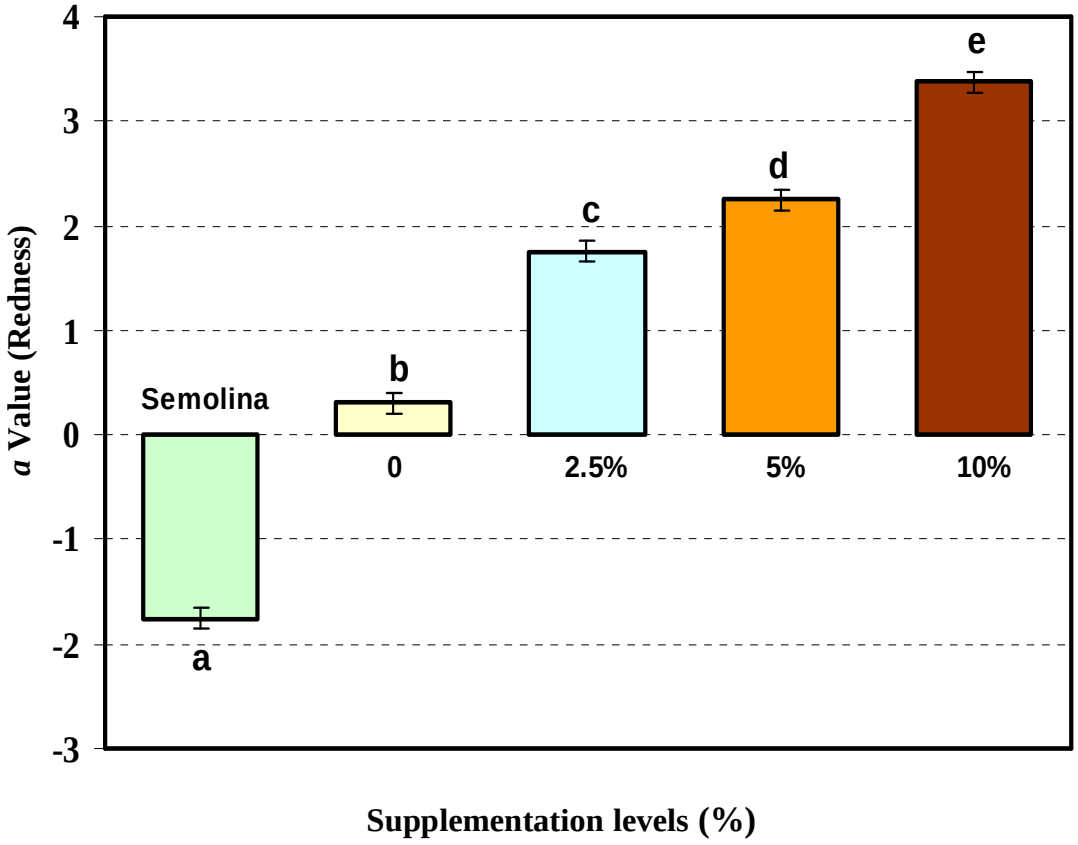


Fig 65. *a* Values of Spaghetti with Different Levels of Supplementation Compared to Semolina

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

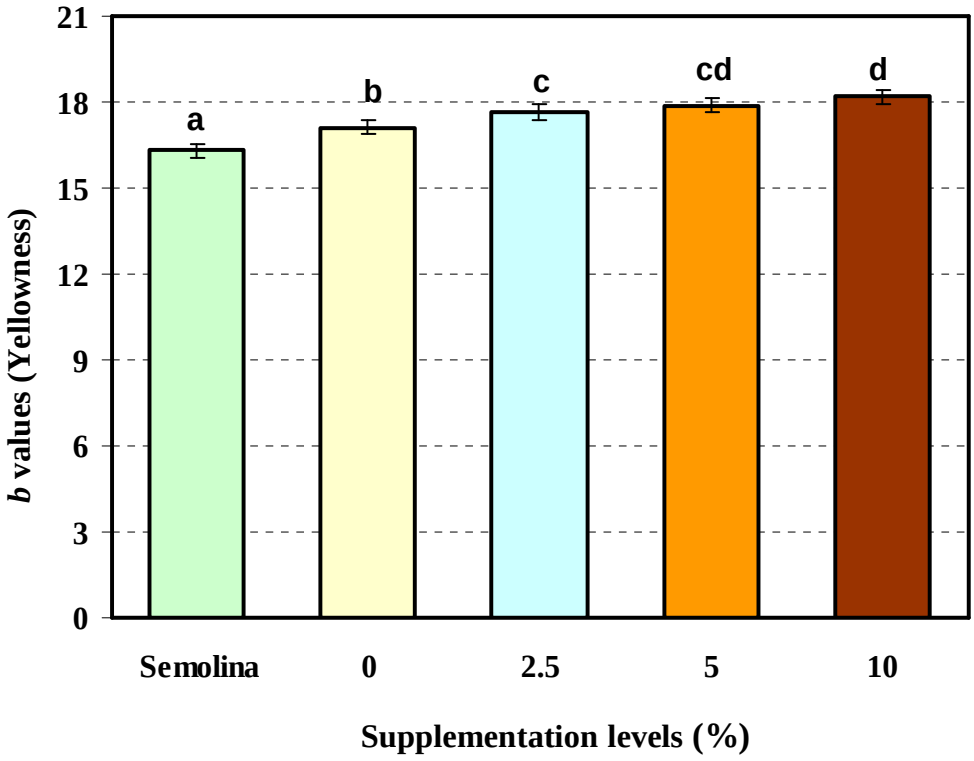


Fig 66. *b* Values of Spaghetti with Different Levels of Supplementation Compared to Semolina

***Values followed by different letters are significantly different (P<0.05) for three determinations**

ePrint@CFTRI

The *L* value of spaghetti was decreased and *b* value increase when pea flour or pea protein concentrate was used at different levels in spaghetti formulation (Nielsen et al., 1980).

Scanning Electron Microscopy of Spaghetti

Food products cannot only be characterized by chemical composition but also by three-dimensional organization at three distinct levels: molecular, microscopic, and macroscopic. The molecular level governs the seric and hindrance and interactions of the food macromolecules. At the microscopic level, the structures and interactions of larger components such as protein, starch, matrices and polymers play a critical role in food texture. At this level, microscopic methods (e.g. optical and scanning electron microscopy) allow the study of the organization of these different components. Such techniques are of particular interest for pasta in which partly swollen starch granules are encapsulated by a protein network. This network is responsible for the slow degradation of starch in pasta, due to its limiting effect on the accessibility of starch to α-amylases (Fardet et al., 1998).

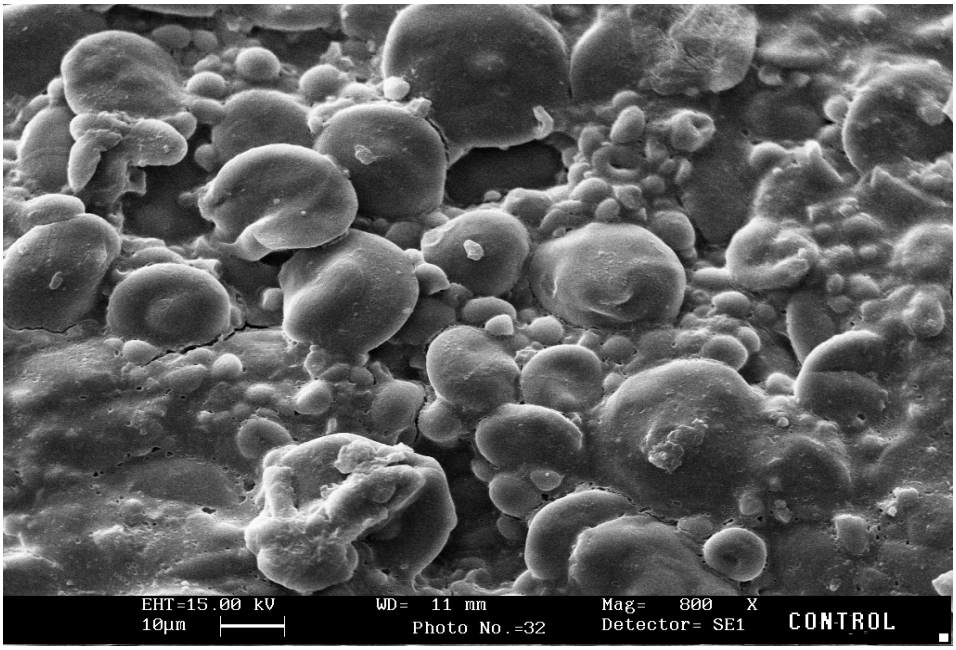
In cereal-based products, a good understanding of the three levels of food organization is essential for the improvement of food product formulation. For example, the addition of surfactants to flour alters the textural appearance of the bread crumb (Pomeranz et al., 1970). Such technological modifications can be characterized by certain rheological measurements (Keetels et al., 1996), but may also be studied by artificial vision associated with image analysis (Bertrand et al., 1992).

ePrint@CFTRI

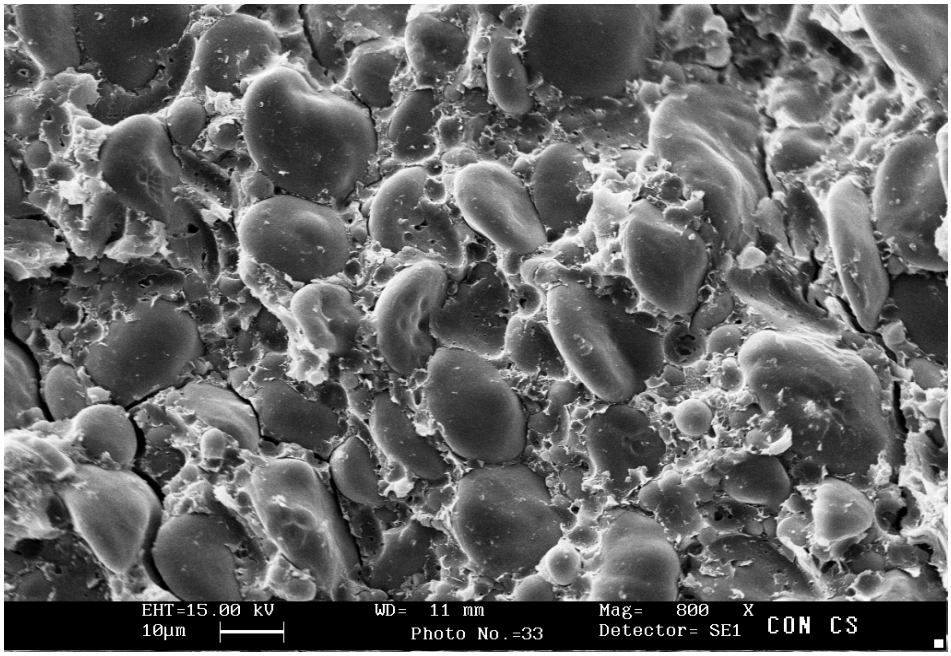
The SEM of surface and cross sections of spaghetti prepared by different levels of supplementation with MPI compared to control spaghetti are shown in Figures 67 to 70. The figures 67a to 70a show numerous starch granules of varying size visible on the surface structure of dry spaghetti. In addition, the outer layer of dry spaghetti appeared to be associated with a thin protein film as already reported in the literature (Dexter et al., 1978; Matsuo et al., 1978; Donnelly, 1982; Cunin et al., 1995). Moreover, many cracks and small holes were apparent in the protein matrix at the surface. This was partly due to shrinkage during sample preparation and partly due to the surface tension in spaghetti dough during drying. Enrichment of semolina with MPI increases the matrix around the starch granules as seen in Figures 67 to 70.

The internal structure of dry spaghetti (Fig 67b to 70b) shows a homogenous and porous structure where starch granules were deeply embedded in a protein matrix. The supplementation of semolina with MPI increased the protein matrix around the starch granules. The low moisture content of pasta dough and the insufficient mixing during extrusion do not allow a complete development of a gluten network as would be the case in bread dough. The results of SEM of spaghetti samples are in close agreement with the results reported by others (Matsuo et al., 1978; Resmini and Pagani, 1983; Pagani et al., 1986).

ePrint@CFTRI



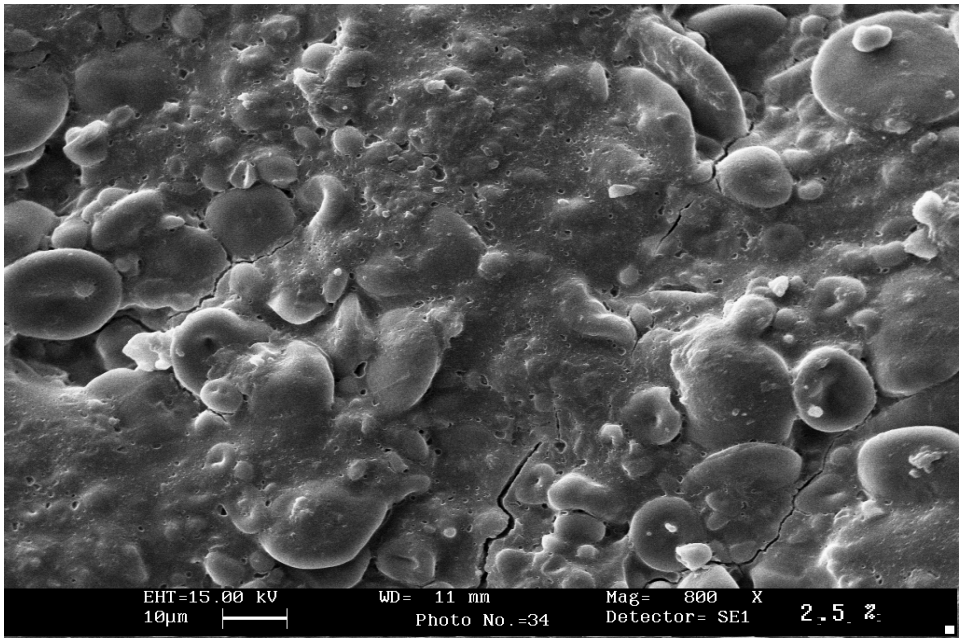
(a)



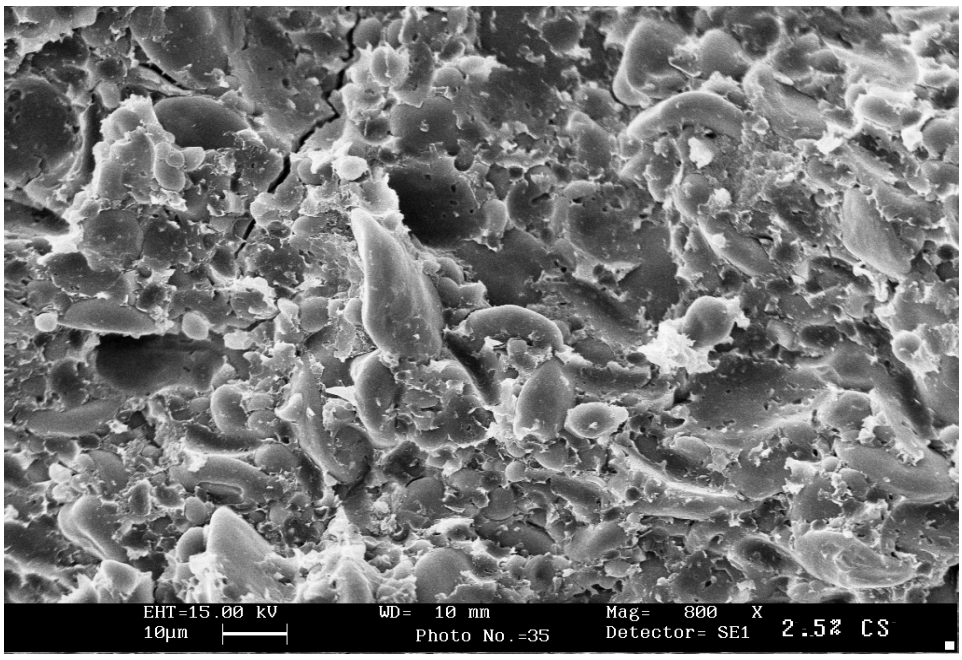
(b)

Fig 67. SEM of Surface (a) and Cross Section (b) of Control Spaghetti

ePrint@CFTRI



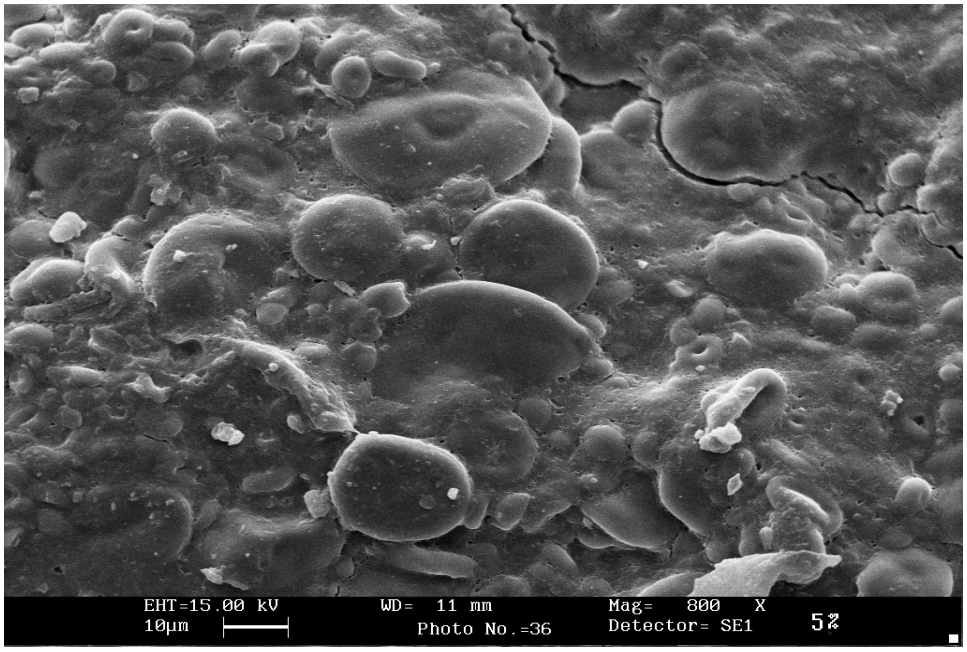
(a)



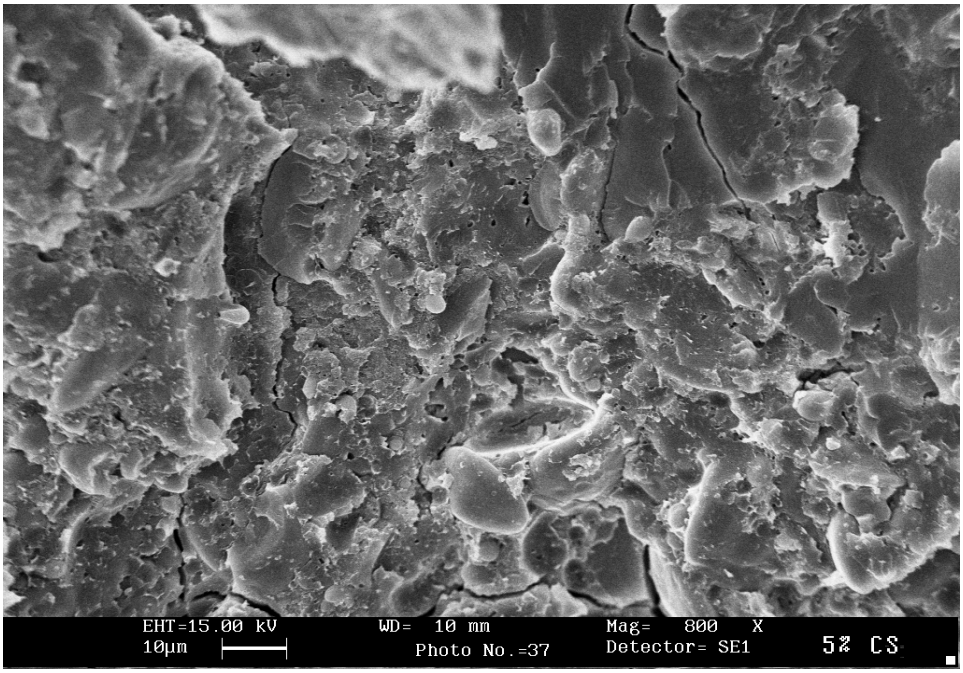
(b)

Fig 68. SEM of Surface (a) and Cross Section (b) of Spaghetti with 2.5% Supplementation

ePrint@CFTRI



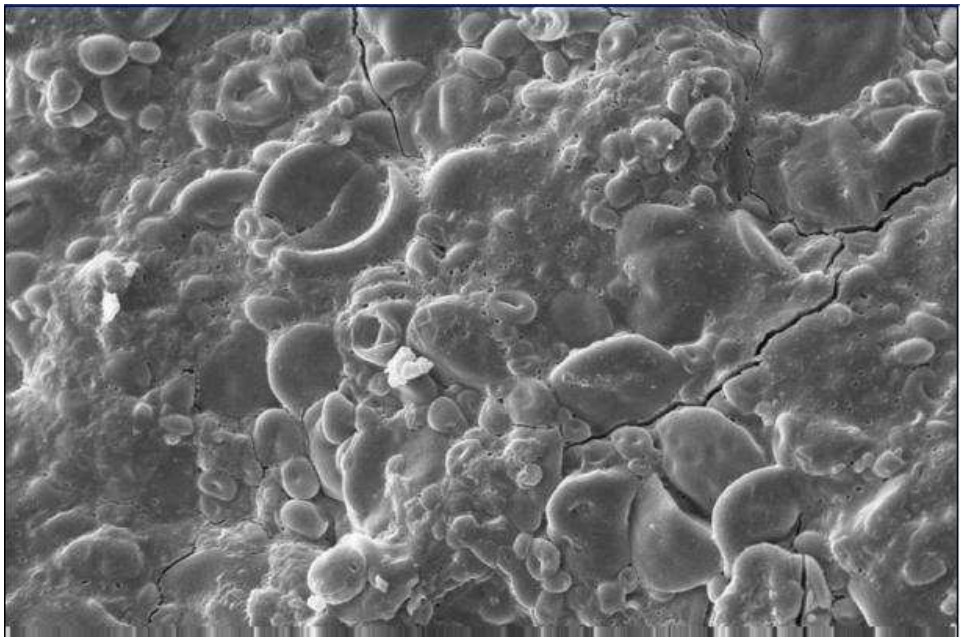
(a)



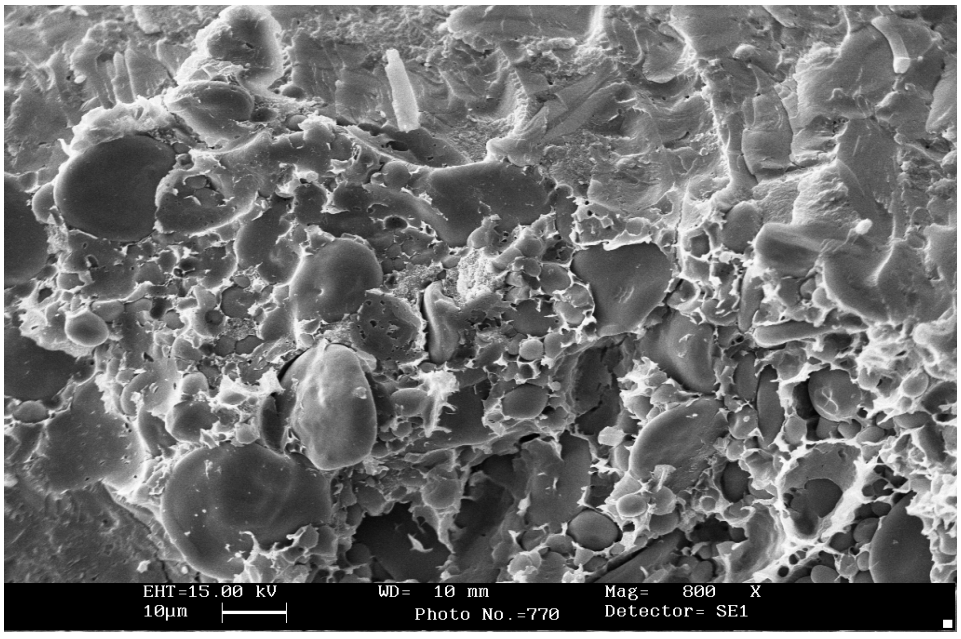
(b)

Fig 69. SEM of Surface (a) and Cross Section (b) of Spaghetti with 5% Supplementation

ePrint@CFTRI



(a)



(b)

Fig 70. SEM of Surface (a) and Cross Section (b) of Spaghetti with 10% Supplementation

ePrint@CFTRI

Chemical Composition of Enriched Spaghetti

The chemical composition of enriched spaghetti compared to control is presented in Table 41. The most pronounced effect of enrichment was increase in protein content. The protein content of control was 11.5% and it increased to 13.8, 16 and 20.7% with Supplementation of semolina with 2.5, 5 and 10% mustard protein isolate, respectively. The increase of protein content was around 40% with supplementation of each 5% mustard protein isolate. All other constituents did not show any significant change.

Cooking Characteristics of Enriched Spaghetti

Control and enriched spaghetti were cooked in boiling water for 10 min. After removal of the water, the cooked spaghetti samples were evaluated for the cooking characteristics (Fig 71 to 74). The cooking characteristics of enriched spaghetti was compared to control and presented in Table 42. The cooked weight of control was 31.4 g and it reduced to 28.2, 27.4, and 26.5g for 2.5, 5 and 10% supplementation, respectively. Nielsen et al. (1980) have reported that the use of higher proportion of denatured protein in enriched spaghetti lowered the cooked weight of samples. Haber et al. (1978) have reported that the cooked weight of spaghetti made from 100% semolina decreased markedly by the addition of high-protein materials from soybean or cottonseed. Similar observation was made by supplementation of spaghetti with different legumes including navy, pinto and lentil (Bahnassey and Khan, 1986).

ePrint@CFTRI

Table 41. Chemical Composition of Spaghetti with Different Levels of Supplementation

Constituents (%)	Supplementation Levels (%)			
	Control	2.5%	5%	10%
Moisture	13.2 ± 0.1	12.0 ± 0.1	11.9 ± 0.1	11.9 ± 0.1
Ash	0.82 ± 0.01	0.81 ± 0.02	0.80 ± 0.02	0.79 ± 0.3
Protein (N×6.25)	11.5 ± 0.1	13.8 ± 0.08	16.0 ± 0.1	20.7 ± 0.1
Crude Fiber	5.05 ± 0.03	4.88 ± 0.02	4.82 ± 0.03	4.71 ± 0.03
Dietary Fiber	8.34 ± 0.02	8.28 ± 0.03	8.23 ± 0.02	8.20 ± 0.03

Mean ± SD of three determinations

ePrint@CFTRI

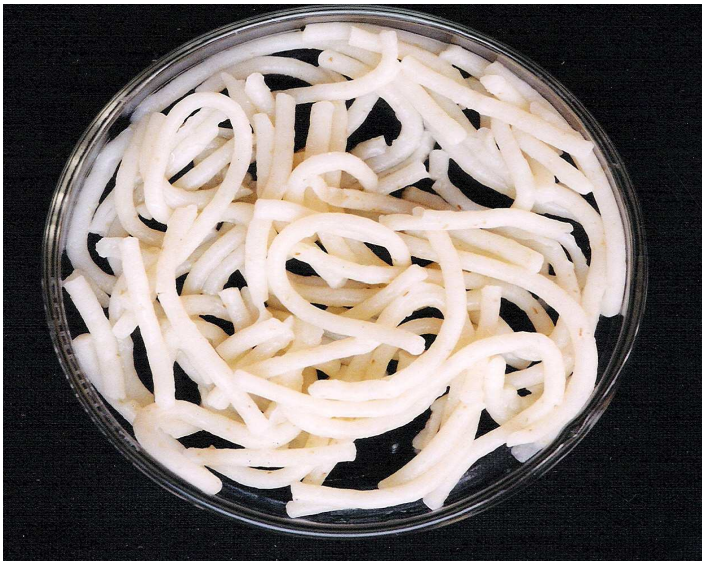


Fig 71. Cooked Spaghetti Control

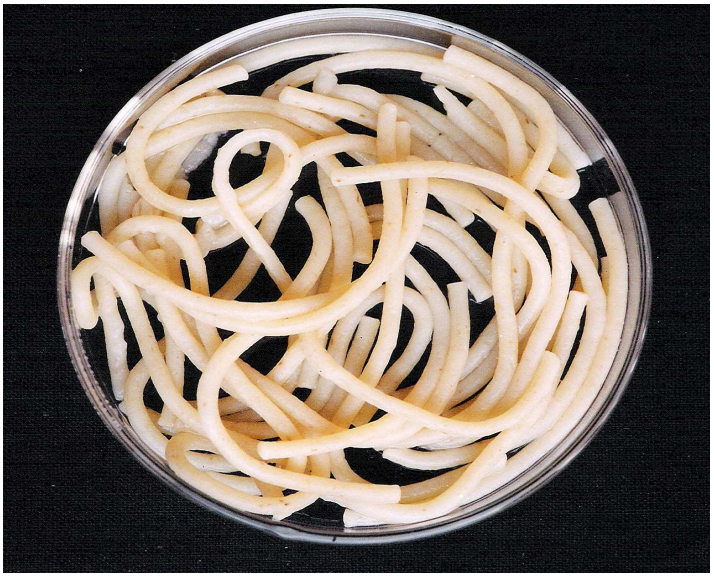


Fig 72. Cooked Spaghetti with 2.5% Supplementation

ePrint@CFTRI

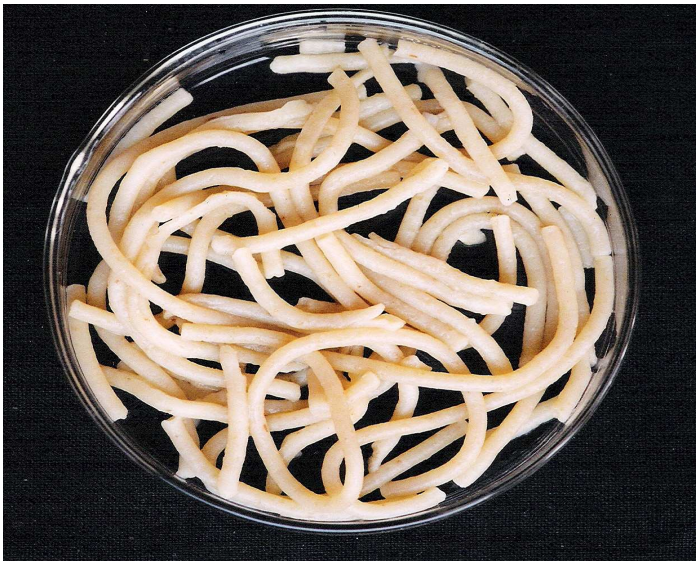


Fig 73. Cooked Spaghetti with 5% Supplementation

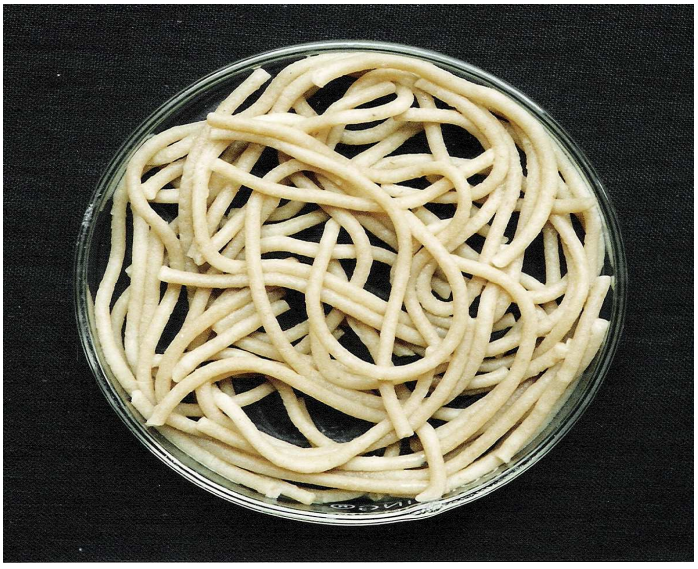


Fig 74. Cooked Spaghetti with 10% Supplementation

ePrint@CFTRI

Table 42. Cooking Characteristics of Spaghetti with Different Levels of Supplementation

Parameters	Supplementation Levels (%)			
	Control	2.5%	5%	10%
Cooked Weight (g)[#]	31.4 ± 0.15[a]	28.2 ± 0.10[b]	27.4 ± 0.2[c]	26.50 ±0.15[d]
Cooking Loss (%)	7.16 ± 0.04[a]	6.65 ± 0.05[b]	6.31 ± 0.04[bc]	6.15 ± 0.05[c]
Protein Loss (%) [+]	11.3 ± 0.12[a]	9.4 ± 0.10[b]	8.1 ± 0.15[c]	7.5 ± 0.10[d]
Firmness (gf)	77 ± 2[b]	90 ± 3[a]	90 ± 2[a]	90 ± 2[a]
Stickiness(N/m^2)	437 ± 10[a]	409 ± 12[b]	395 ± 8[bc]	378 ± 9[c]

[#] From 10 g Raw Spaghetti

[+] As Percent of Total Protein

*Mean ± SD of three determinations

**Values Followed by different letters for each Cooking Characteristic are significantly different ($p<0.05$)

ePrint@CFTRI

Breen et al. (1977) have also reported that the cooked weight of spaghetti made from a bean formula was lower than that of control.

The results of cooking loss and protein loss are presented in Table 42. As the level of enrichment increased, the cooking loss and protein loss were decreased. The decrease may be due to low solubility of mustard protein isolate that resulted in lowering the cooking loss and protein loss in the enriched spaghetti compared to control.

Table 42 shows the results of firmness of enriched spaghetti samples as compared to control. The firmness value of control was 77 gf which increased to 90 gf for all the supplementation levels. There was no significant difference between the firmness values of enriched spaghetti samples. Haber et al. (1978) reported that addition of high-protein materials from soy and cotton seed to semolina resulted in increase in the firmness of spaghetti. Similar observations have been made by others (Paulsen, 1961; Matsuo et al., 1972; Bahnassey and Khan, 1986).

The stickiness of control spaghetti was 437 N/m^2 and it reduced to 409, 395 and 378 N/m^2 for 2.5, 5 and 10% enrichment levels, respectively (Table 42). The reduction in the stickiness may be due to reduction in starch proportion in the enriched spaghetti or physical entrapment of starch in protein network with increased supplementation level. These results are in close agreement with increased protein matrix around starch granules observed in SEM of enriched spaghetti samples (Fig 68 to 70).

ePrint@CFTRI

Sensory Evaluation

The sensory characteristics of cooked control and enriched spaghetti samples were evaluated for different parameters like color and appearance, taste and flavor, texture, mouth feel and overall quality (Fig 75 and 76). The results showed that there was no significant difference in color preference of panelist between control and 2.5% enriched spaghetti. However 5% and 10% enrichment scored higher values. This is mainly due to higher *b* values (yellowness) of the enriched spaghetti (Fig 66) compare to control. Generally, the higher yellow color in pasta products is highly accepted by the consumers.

The flavor and texture of control and 2.5% enriched spaghetti did not show any significant difference (Fig 75). However, the spaghetti samples enriched with 5% and 10% MPI scored lower for taste and flavor. This may be due to lower wheaty flavor of enriched spaghetti compared to control. The mouthfeel of control and 2.5% enriched spaghetti did not show any significant difference (Fig 75). On the other hand, mouth feel of 5 and 10% enriched samples were less preferred by the panelists.

The texture of enriched spaghetti samples were scored higher than the control sample (Fig 75). This could be due to higher firmness and lower stickiness of enriched spaghetti compared to control (Table 42). The overall quality of enriched spaghetti was higher than control (Fig 76) mainly due to good texture and color. All the panelists showed higher preference for enriched spaghetti.

ePrint@CFTRI

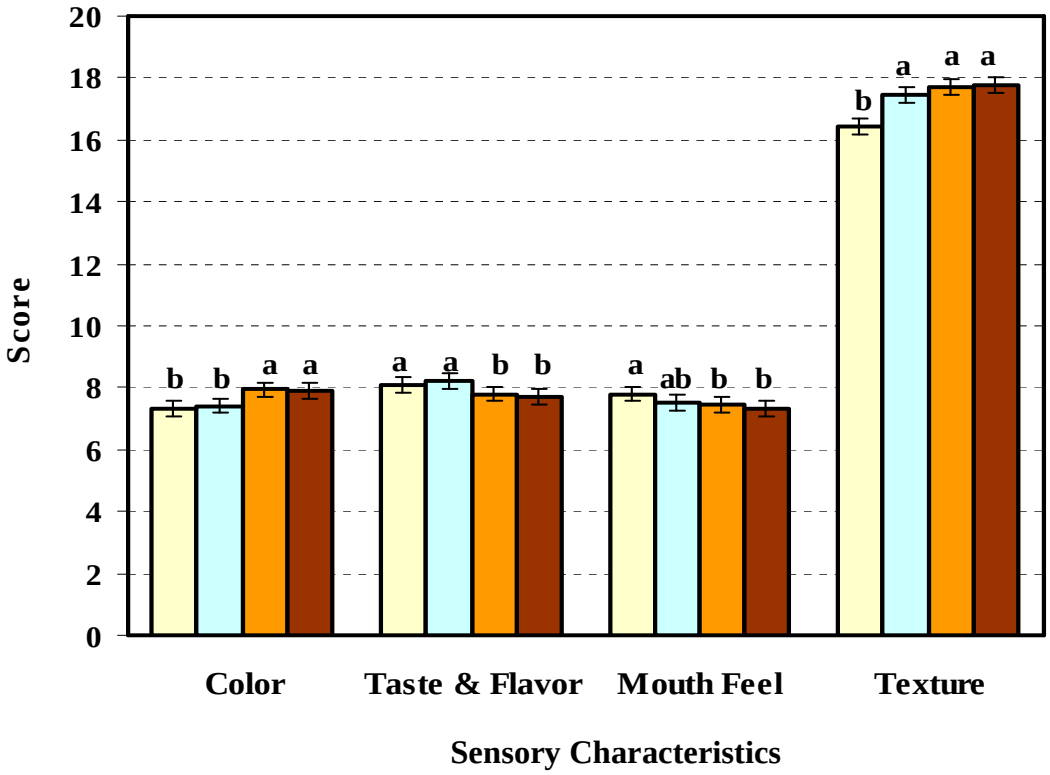


Fig 75. Sensory Evaluation of Spaghetti with Different Levels of Supplementation

Control 2.5% 5% 10%

***Values followed by different letters for each Sensory Characteristics are significantly different (P<0.05)**

ePrint@CFTRI

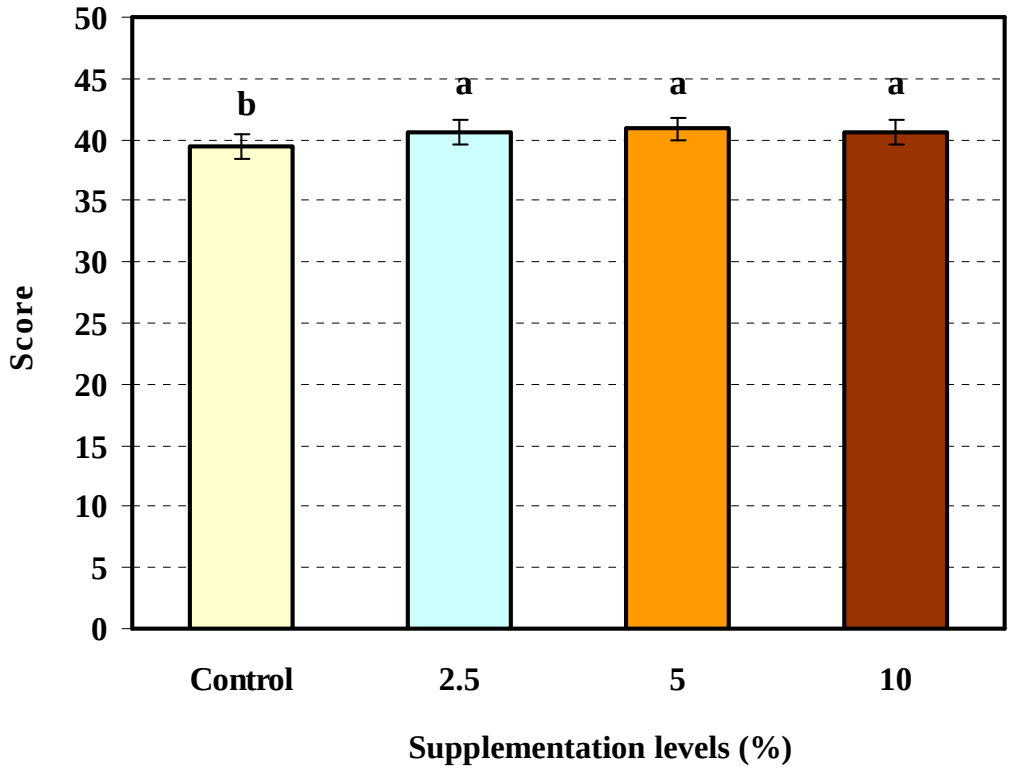


Fig 76. Overall Quality of Spaghetti with Different Levels of Supplementation

***Values followed by different letters are significantly different (P<0.05)**

ePrint@CFTRI

Nutritional Evaluation

One of the great challenges today is to develop inexpensive foods that are nutritionally superior and at the same time acceptable to the intended consumer. Wheat is abundant in some area of the world and is one of the less expensive cereals available for creating fabricated foods high in nutrition.

Cereal and cereal products are widely consumed as a source of energy as well as protein intake. Macaroni products, produced mainly from hydrated semolina, durum flour, farina or any combination of two or more of those. Macaroni products are inexpensive, easy to prepare, shelf stable and can be served in different ways. The main cereal used in the pasta products is wheat which is deficient in the essential amino acid lysine and to a lesser extent threonine and methionine. To improve the nutritional value of cereal based food products different approaches have been used viz. enrichment of cereal products with various nutrients, high protein ingredients, amino acids, minerals and vitamins. Fortification of existing foods such as bread and pasta with protein and minerals are very common. To improve the nutritional quality of the final pasta products further addition of high quality protein sources (milk powder, whey and fish flour) and sources rich in protein such as soy flour, soy protein concentrate and isolate have been used **(**Paulsen, 1961; Kwee et al, 1969; Hanna et al., 1978; Schoppet et al., 1976; Haber et al., 1978; Morad et al., 1980; Beslagic, 1981; Bahnassey and Khan, 1986**).**

There is an increasing interest among vegetarians and health conscious people to consume protein enriched foods from plant sources, which have no cholesterol and low saturated fat content in general.

ePrint@CFTRI

The amino acid composition of control and enriched spaghetti samples prepared by supplementation of semolina with different levels of MPI are presented in Table 43. The results showed that the supplementation of semolina with MPI generally increased the amount of all the amino acids. However, the amount of glutamic acid, proline and phenylalanine reduced with enrichment of semolina with MPI. This may be due to the presence of higher amounts of these amino acids in semolina. Enrichment of semolina with MPI had pronounced effect on lysine, cysteine, arginine and histidine contents. This is possibly due to higher contents of these amino acids in MPI (Chapter 4, Table 34).

The nutritional indices of control spaghetti compared to spaghetti prepared by enrichment of semolina with different levels of MPI are presented in Table 44. The chemical score indicated that lysine was the first limiting amino acid in all the cases including the control. The computed PER (C-PER) was increased from 0.45 for control to 1.0, 1.58 and 1.92 for 2.5, 5 and 10% enrichment, respectively indicating that mustard proteins are good source of essential amino acids. The PDCAAS score for control spaghetti was 59.7 which increased to 79.5, 100 and 100 for adults by enrichment of semolina with 2.5, 5 and 10% MPI, respectively. The results clearly indicated that mustard protein isolate could be used as a good source of protein in high protein formulation for adults. In general, enrichment with MPI improved all the nutritional quality of the spaghetti samples.

ePrint@CFTRI

Table 43. Amino Acid Composition of Spaghetti with different Levels of Supplementation (g/100 g protein)

Amino Acids	Supplementation Levels (%)			
	Control	2.5 %	5 %	10 %
Lysine	1.80 ± 0.03	2.43 ± 0.01	3.10 ± 0.03	3.75 ± 0.04
Methionine	1.38 ± 0.03	1.53 ± 0.03	1.76 ± 0.01	2.02 ± 0.02
Cysteine	1.21 ± 0.05	1.41 ± 0.04	1.76 ± 0.04	2.12 ± 0.03
Tryptophan	1.69 ± 0.04	1.66 ± 0.04	1.65 ± 0.05	1.63 ± 0.06
Histidine	1.15 ± 0.05	1.50 ± 0.05	1.76 ± 0.04	2.39 ± 0.03
Isoleucine	2.88 ± 0.07	3.16 ± 0.06	3.25 ± 0.03	3.32 ± 0.04
Leucine	8.56 ± 0.04	8.43 ± 0.05	8.29 ± 0.06	8.12 ± 0.03
Threonine	2.00 ± 0.03	2.35 ± 0.05	2.80 ± 0.06	3.23 ± 0.05
Valine	3.86 ± 0.04	4.17 ± 0.03	4.28 ± 0.05	4.38 ± 0.06
Phenylalanine	5.56 ± 0.02	5.38 ± 0.03	5.15 ± 0.05	4.76 ± 0.04
Tyrosine	1.59 ± 0.01	1.70 ± 0.02	1.74 ± 0.02	1.89 ± 0.03
Aspartic acid	3.02 ± 0.05	3.43 ± 0.07	3.89 ± 0.06	4.78 ± 0.08
Glutamic acid	34.61 ± 0.25	31.28 ± 0.18	28.67 ± 0.32	25.30 ± 0.25
Serine	4.91 ± 0.10	4.75 ± 0.08	4.69 ± 0.05	4.55 ± 0.05
Glycine	2.91 ± 0.04	3.26 ± 0.04	3.37 ± 0.03	3.55 ± 0.05
Arginine	4.30 ± 0.06	5.37 ± 0.04	6.15 ± 0.05	7.07 ± 0.08
Alanine	3.35 ± 0.05	3.65 ± 0.05	3.97 ± 0.03	4.25 ± 0.05
Proline	15.22 ± 0.15	14.54 ± 0.18	13.72 ± 0.20	12.89 ± 0.14

Mean ± SD of four determinations

ePrint@CFTRI

Table 44. Nutritional Indices of Spaghetti with Different Levels of Supplementation

Parameters		Supplementation Levels (%)			
		Control	2.5%	5%	10%
In vitro digestibility (%)*		83 ± 0.5	84.7 ± 0.5	87.1 ± 0.4	88.6 ± 0.4
Chemical Score		47.3	60.5	73.6	85.4
Limiting Amino Acid		Lysine	Lysine	Lysine	Lysine
C-PER		0.45	1.00	1.58	1.92
EAAI		53.8	62.8	66.5	75.3
P-BV		47	56.8	60.8	70.4
NI		6.2	7.9	9.8	14.6
PDCASS	10-12 years old	34	46.8	61.4	75.5
	Adults	59.7	79.5	100	100

*Mean ± SD of three determinations

ePrint@CFTRI

Mustard/rapeseed forms one of the major oilseeds of world and India. Most of seeds are used for oil extraction and the defatted cake which is a good source of protein being used for animal feed or fertilizer. The use of mustard/rapeseed protein as a source of protein is limited by the presence of undesirable and toxic anti-nutritional compounds such as glucosinolates, phenolics, phytates, and hull. Number of methods has been reported to isolate protein and to reduce the toxic and anti-nutritional constituents. However, none of these methods completely eliminate all the anti-nutritional and toxic constituents.

In the present investigation, the mustard variety T-59 which is grown on a large scale in India was used for the preparation of dehulled defatted meal. In addition, a commercial variety of mustard was used to prepare cake by Ghani pressing. The cake after defatting was fractionated to obtain low hull and high protein fraction. The dehulled meal and low hull fraction of cake were used for this study. A method was developed for the isolation and detoxification of protein from dehulled meal/low hull fraction of cake. The effect of isolation and detoxification method on the chemical composition, physicochemical, functional, and nutritional properties of protein has been studied.

The detoxified protein isolate was used in the preparation of high protein spaghetti. The effects of supplementation of semolina with mustard protein isolate on the chemical composition, cooking characteristics, color, and nutritional quality of spaghetti samples were evaluated.

ePrint@CFTRI

The following results were obtained and conclusions drawn.

- Dehulling, defatting, size reduction and air classification reduced the crude fiber content by 70% and increased the protein content from 22 to 48% in comparison to whole mustard seeds.

- Fractionation of defatted commercial cake after size reduction and separation with Quadromat Mill® resulted in three fractions with different size and hull contents. Defatting, size reduction and separation reduced the crude fiber by 60% and increased the protein content by 28% compared to cake.

- A process has been developed for isolation and detoxification of protein from meal/cake involving alkaline extraction, activated carbon treatment, heat coagulation, washing and spray drying.

- The process resulted with a yield of 60%, and the protein isolate obtained has a protein content of 95% and negligible amounts of carbohydrate, fiber and ash. The protein isolate was light in color and bland to taste.

- The process reduced isothiocyanate by 99.5% and completely removed the oxazolidinethione content in protein isolate. Trypsin inhibitor activity in the protein isolate was negligible compared to meal. Phytate and phenolics content were reduced by 99 and 98.7%, respectively.

- The isolation and detoxification of protein from low hull fraction of cake resulted in a protein concentrate with a protein content of 80% and yield was around 58%.

ePrint@CFTRI

- The hydrolyzed products of glucosinolates, isothiocyanate and oxazolidinethione reduced by 98.6 and 99.1%, respectively in the protein concentrate. The phytate and phenolics were removed by 97.5 and 98%, respectively. The trypsin inhibitor activity was completely inactivated.

- The protein isolate/concentrate showed low solubility at different pH values. Modification of protein isolate by enzyme alcalase improved the solubility from 25-40% to 60-75% whereas in the protein concentrate, the solubility improved from 20-35% to 50-65% on hydrolysis.

- The protein isolate/concentrate showed lower Water Absorption Capacity (WAC) and Fat Absorption Capacity (FAC) compared to meal/low hull fraction of cake. However enzyme modification improved the WAC and FAC.

- The Emulsion Capacity (EC), Foam Capacity (FC) and Foam Stability (FS) of protein isolate/concentrate were lower than meal/low hull fraction of cake. However enzyme hydrolysis improved the EC and FC of protein hydrolysates but reduced the FS drastically.

- The color characteristics (*L*, *a*, and *b* values) of protein isolate were lower than dehulled meal. However, use of ascorbic acid during the isolation process resulted in protein isolate with better color characteristics compared to isolate prepared without ascorbic acid.

ePrint@CFTRI

- The *L* value (Lightness) of protein concentrate was higher than that of the low hull fraction of cake. This could be due to removal of phenolics during the preparation of protein concentrate.

- The Scanning electron Microscopy (SEM) of meal, low hull fraction of cake, protein isolate and concentrate showed spherical particles (protein bodies) 1-20 μm in diameter. The particle size of spherical particles in protein hydrolysates were around 1-10 μm and many of them were less than 5 μm in diameter.

- The SDS-PAGE pattern of meal, low hull fraction of cake, protein isolate and concentrate showed three major bands corresponding to molecular weight of 34,000; 28,000; and 20,000 Da. However, the protein hydrolysates did not show any band corresponding to the above subunits due to the breakdown of these proteins by the enzyme.

- The SDS-PAGE pattern of different fractions obtained from the commercial cake did not show any difference. However, the patterns were similar to those of dehulled meal proteins.

- The amino acid profile of protein isolate showed higher amounts of leucine, methionine and cysteine and lower levels of lysine compared to the meal. However, the amino acid composition of protein hydrolysate prepared from meal did not show any difference compared to the protein isolate prepared from meal.

- The *in vitro* digestibility and the calculated nutritional indices such as Computed Protein Efficiency Ratio (C-PER), Essential Amino Acid Index (EAAI), Predicted Biological Value (P-BV),

ePrint@CFTRI

Nutritional Index (NI), and PDCAAS of protein isolate and hydrolysate were higher than the dehulled defatted meal.

- Protein isolate showed higher C-PER, EAAI, Predicted Biological Value, Nutritional Index, compared to casein and the PDCAAS value for age group of 10-12 years old and adults were comparable to casein.

- The protein from low hull fraction of cake contained higher amounts of lysine and tryptophan, while the protein concentrate and hydrolysate contained higher amounts of leucine, isoleucine, cysteine, phenylalanine and tyrosine.

- The protein concentrate and hydrolysate from cake showed higher *in vitro* digestibility, Chemical Score, Computed Protein Efficiency Ratio, Essential Amino Acid Index, Predicted Biological Value, Nutritional Index, and PDCAAS compared to low hull fraction of cake.

- The spaghetti prepared by supplementation of semolina with 2.5, 5 and 10% mustard protein isolate showed higher protein content compared to control spaghetti.

- The enriched spaghetti samples prepared by supplementation of semolina with mustard protein isolate had greater firmness and lower stickiness values. The cooking loss, protein loss and cooked weight were decreased with increased supplementation levels.

ePrint@CFTRI

- The *L* values (lightness) of spaghetti decreased with increased supplementation levels. On the other hand, *b* values (yellowness) and *a* values increased with increased supplementation levels.

- The results of the sensory evaluation of spaghetti samples indicated that the color and texture scores were higher, while the taste and mouthfeel were lower with increased supplementation levels. Overall quality of all the spaghetti prepared with mustard protein isolate scored higher than control spaghetti.

- Supplementation of spaghetti with mustard protein isolate generally increased the essential amino acid composition of enriched products compared to control spaghetti.

- All the nutritional indices of enriched spaghetti prepared with mustard protein isolate were higher than the control spaghetti.

- Scanning Electron Microscopy (SEM) of spaghetti samples prepared with mustard protein isolate showed increased protein matrix around the starch granules.

In conclusion:

The mustard protein isolate prepared by the improved method with reduced anti-nutritional constituents could be exploited for commercial production. The protein isolate with improved nutritional characteristics could also be used as a good source of protein in high protein food formulations for adults.

ePrint@CFTRI

Aalami, M., Leelavathi, K. (2006). Physicochemical properties and spaghetti making quality of Indian durum wheat (A thesis to be submitted). University of Mysore, India.

Adler-Nissen, J. (1979). Determination of the degree hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J. Agric. Food Chem*., **27**, 1256.

Adler-Nissen, J. (1981). Limited enzymic degradation of proteins- a new approach in the industrial application of hydrolyses. *J. Chem. Technol. Biotechnol*., **32**, 138.

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*. Elsevier, London.

Adler-Nissen, J., and Olsen, H. (1979). The influence of peptide chain length on taste and functional properties of enzymatically modified soy protein. *ACS Symp. Ser*., **92**, 125.

Adler-Nissen, J., Erikson, J., and Olsen, H. (1983). Improvement of the functionality of vegetable proteins by controlled enzymatic hydrolysis. *Plant Food Hum. Nutr.*, **32**, 411.

Adrian, J. (1974). Nutritional and physiological consequences of the millard reaction. *World Rev. Nutr. Diet.*, **19**, 71.

Afzalpurkar, A.B., Mukherjee, K.D., and Mangold, H.K. (1974). Detoxification of rapeseed meal. In *proceeding, 4th international rapeseed conference*, Giessen, Germany, PP: 609-614.

Ahmed, Z. A. (1972). Naturally occurring glucosinolates with special reference to those of family Capparidaceae. *Planta. Med*., **21**, 35.

Ahuja, K.L. (1994). Oil quality and modification of fatty acid composition. In *Group Discussion on Oilseed Quality*, Directorate of Oilseeds Research, Rejinderanagar, Hyderabad.

Akeson, W.R., and Stahman, M.A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr.,* **83**, 257.

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.

ePrint@CFTRI

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*., (In Press).

Altschul, A.H., and Wilcke, H.L. (1985). *New Protein Foods*. Academic Press, Inc., New York, USA.

Altschul, A.M. (1974). *New Proteins Foods.* Vol. IA, Academic Press, New York, NY.

Anand, I.J., Malik, R.S., and Rawat, D.S. (1976). Breeding rapeseed mustard oil crops for productivity and improved quality. In *Proceeding Symposium on Rapeseed and Mustard*, PP: 58-70, Mysore, India.

Anderson, A.H. (1946). The pharmacology of activated charcoal. 1. Adsorption power of charcoal in aqueous solutions. *Acta Pharmacol. Toxicol*., **2**, 69.

Anderson, G.H., Li, G.S.K., Jones, J.D., and Bendar, F. (1975). Effect of hydrogen peroxide treatment on the nutritional quality of rapeseed flour fed to weanling rats. *J. Nutr*., **105**, 317.

Anderson, R.J. (1914). A contribution to the chemistry of phytin. *J. Biol. Chem*., **17**, 171.

Anon. (1980). Volatile oil in mustard seed. Official final action 30. 027. *Official Method of Analysis of the Association of Official Analytical Chemists*, 13th Edn., 499.

AOAC. (1990). Protein efficiency ratio rat bioassay. In *Official Methods of Analysis of Association of Official Analytical Chemists*.15th Edn., AOAC International.

Appleqvist, L.A. (1971). Composition of seeds of cruciferous oil crops. *J. Am. Oil Chem. Soc*., **48**, 851.

Appleqvist, L.A., and Josefsson, E. (1967). Method of quantitative determination of isothiocyanates and oxazolidinethione in digests of seed meals of rape and turnip rape. *J. Sci. Food Agric*., **18**, 510.

ePrint@CFTRI

Appleqvist, L.A., and Ohlson, R. (1972). *Rapeseed: Cultivation, Composition, Processing and Utilization*. Elsevier Pub. Co., Amsterdam.

Arai, S., Yamashita, M., and Fujimaki, M. (1975). Plastein reaction and its applications. *Cereal Food World.*, **20**, 107.

Arguello, L.G., Sensharma, D.K., Qiu, F., Nurtaeva, A., and El-Rassi, Z. (1999). High-performance liquid-phase separation of glycosides. Analytical and micro-preparative HPLC combined with spectroscopic and enzymatic methods for generating a glucosinolate library. *J. AOAC Int*., **82**, 1115.

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.

Arzu, A., Mayorga, M., and Rolcz, C. (1972). Enzymatic hydrolysis of cotton seed protein. *J. Agric. Food Chem*., **20**, 805.

Aspinall, G.O., and Jiang, K.S. (1974). Rapeseed hull protein. *Carbohyd. Res*., **38**, 247.

Association of Official Analytical Chemists (AOAC). (1984). *Official Methods of Analysis,* Williams, 14th Edn., Arlington V.A., USA. PP: 162-218.

Association of Official Analytical Chemists (AOAC). (2000). *Official Methods of Analysis of AOAC International*, methods 934.01, 988.05, 920.39, 942.05, 962.09. Arlington, VA, USA: AOAC International.

Bahnassey, Y., and Khan, K. (1986). Fortification of spaghetti with edible legumes. II. Rheological processing and quality evaluation studies. *Cereal Chem*., **63**, 216.

Bahnassey, Y., Khan, K., and Harrold, R. (1986). Fortification of spaghetti with edible legumes. I. Physicochemical, anti-nutritional, amino acid and mineral composition. *Cereal Chem*., **63**, 210.

Ballester, D., Rodrigo, R., Nakouzi, J., Chichester, C.D., Yanez, E., and Monckeberg, F. (1970). Rapeseed meal. III. A simple method for detoxification. *J. Sci. Food Agric*., **21**, 143.

ePrint@CFTRI

Ballester, D., Rodrigo, R., Nakouzi, J., Chichester, C.O., Yanez, E., and Monckeberg, F. (1970). Rapeseed meal. II. Chemical composition and biological quality of protein. *J. Sci. Food Agric*., **21,** 140.

Ballester, D., Rodriguez, B., Rojas, M., Brunser, O., Reid, A., Yanez, E., and Monckeberg, F. (1973). Rapeseed meal. IV. Continuous water extraction and short-term feeding studies in rats with the detoxified production. *J. Sci. Food Agric*., **24**, 127.

Banasik, O.J. (1975). Protein enrichment of pasta products. *Cereal Food World*, **20,** 480.

Barker, F.S., Miller, C.E., Repik, A.J., and Tolles, E.D. (1992). Activated carbon. *Kirk-Othmer Encyclopedia of Chemical Technology*, **4,** 1015.

Barker, L.D., Martens, R.W., and Murray, E.D. (2005). Production of oilseed protein isolate. *U.S. Patent* 200550165220.

Bell, J. (1993). Factors affecting the nutritional value of canola meal: a review. *Can J. Anim. Sci.*, **73,** 679.

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

Bell, J.M., and Shires, A. (1982). Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. *Can. J. Anim. Sci*., **62**, 557.

Bell, J.M., Shires, A., Blake, J.A., Campbell, S., and McGregor, D.I. (1981). Effect of alkali treatment and amino acid supplementation on the nutritive value of yellow and oriental mustard meal for swine. *Can. J. Anim. Sci*., **61**, 783.

Bell, J.M., Youngs, C.G., and Sallans, H.B. (1970). Treatment of rapeseed meal. *Canadian Patent* 839, 653.

Belzile, R.J., and Bell, J. M. (1956). Growth depressing factors in rapeseed oil meal. VII. Effect of myrosinase activity on toxicity following treatments with buffered solutions. *Can. J. Anim. Sci*., **46,** 165.

ePrint@CFTRI

Belzile, R.J., Bell, J.M., and Wetter. L.R. (1963). Growth depressing factors in rapeseed oil meal. V. The effects of myrosinase activity on the toxicity of the meal. *Can. J. Anim. Sci*., **43**, 169.

Bender, A.E. (1972). Processing damage to protein food. A review. *J. Food Technol*., **7**, 239.

Bertrand, D., Le Guerneve, C., Marion, D., Devaux, M.F., and Robert, P. (1992). Description of the textural appearance of bread crumb by video image analysis. *Cereal Chem*., **69**, 257.

Beslagic, S. (1981). Replacement of egg and milk with soy protein in pasta. *J. Am. Oil Chem. Soc.*, **58**, 535.

Betschart, A.A. (1974). Nitrogen solubility of alfalfa protein concentrate as influenced by various factors. *J. Food Sci*., **39**, 1110.

Beuchat, L.R., Cherry, J.P., and Quinn, M.R. (1975). Physicochemical properties of peanut flour as affected by proteolysis. *J. Agric. Food Chem*., **23**, 616.

Bhagya, S., and Sastry, M.C.S. (2003). Chemical, functional and nutritional properties of wet dehulled niger (*Guizotia abyssinica Cass*) seed flour. *Lebensm. Wiss. U. Technol.*, **36**, 703.

Bhagya, S., and Srinivasan, K.S. (1989). Effect of different methods of drying on the functional properties of enzyme treated groundnut flour. *Lebensm. Wiss. U. Technol*., **22**, 329.

Bhatty, R. S. (1972). A note on trichloroacetic acid precipitation of oilseed proteins. *Cereal Chem.,* **49**, 729.

Bhatty, R. S., and Finlayson, A. J. (1973). Extraction of non-protein nitrogen from oilseed meals with different solvents. *Cereal Chem.,* **50**,329.

Bhatty, R.S., MacKenzie, S.L., and Finlayson, A.J. (1968). Proteins of rapeseed (*Brassica napus*) soluble in salt solutions. *Can. J. Biochem*., **46**, 1191.

Bhatty, R.S., Sosulski, F.W., and Youngs, C.G. (1972). Extraction of glucosinolates from rapeseed. *Can. Inst. Food Sci. Technol. J*., **5**, 149.

ePrint@CFTRI

Bidlingmeyer, B.A., Cohen, S.A., and Tarvin, T.L. (1984). Rapid analysis of amino acid using pre-column derivatization. *J. Chromatogr*., **336**, 93.

Birch, G.G., Parker, K.J., and Worgan, J.T. (1976). *Food from waste*. Applied Science Publishers Ltd., London.

Bjergegaard, C., Eggum, B.O., Jensen, S.K., and Sorensen, H. (1991). Dietary fibers in oilseed rape: Physiological and anti-nutritional effects in rats of isolated IDF and SDF added to a standard diet. *J. Anim. Physio. Anim. Nutr*., **66**, 69.

Bjork, I., Liljeberg, H., Ostman, E. (2000). Low glycemic index foods. *Br. J. Nutr*., **83**, 5149.

Bjorkman, R. (1973). Interaction between proteins and glucosinolate, isothiocyanates and oxazolidinethione from *Brassica napus* seed. *Phytochem.*, **12**, 1585.

Bjorkqvist, B., and Hase, A. (1988). Separation and determination of intact glucosinolates in rapeseed by high-performance liquid chromatography. *J. Chromatogr*., **435**, 501.

Blaicher, F.M., Elstner, F., Stein, W., and Mukherjee, K.D. (1983). Rapeseed protein isolates: effect of processing on yield and composition of protein. *J. Agric. Food Chem.*, **31**, 358.

Bobalik, J.M., and Taranto, M.V. (1980). The effect of enzymic modification on the foaming, water absorption and baking quality of defatted soy flour. *J. Food Technol.*, **15**, 637.

Bockelmann, I., Von Dejmek, P., Eriksson, G., and Hallstrom, B. (1977). Potential applications in food processing. In *Reverse Osmosis and Synthetic Membranes*. PP: 445-458. National Research Council, Ottawa, Canada (Food Sci, Technol Abstr. 10, 2A 86, 1978).

Bodwell, C.E., and Hopkins, D.T. (1985). Nutritional characteristics of oilseed proteins. In *New Protein Foods*. Altschul, A.M., and Wilcke, H.L. (Eds.), P.221.

ePrint@CFTRI

Bodwell, C.E., Satterlee, L.D., and Hackler, L.R. (1980). Protein digestibility of the same protein preparations by human and rat assays and by *in vitro* enzymic digestion methods. *Am. J. Clin. Nutr*., **33**, 677.

Bones, A.M., and Rossiter, J.T. (1996). The myrosinase-glucosinolate system, its organization and biochemistry. *Physiol. Plant.*, ***97,*** 194.

Breen, M.D., Banasik, O.J., and Walsh, D.E. (1977). Use of various protein sources in pasta. *Macaroni J*., **26**, 34.

Brooks, J.R., and Morr, C.V. (1982). Phytate removal from soybean protein isolates using ion-exchange processing treatments. *J. Food Sci.,* **47**, 1280.

Cameron, J.J., and Meyers, C.D. (1982). Novel protein isolation procedure. *U.S. Patent* 4,366,097.

Cameron, J.J., and Myers, C.D. (1983). Rapeseed protein isolate. *U.S. Patent* 4, 418, 013.

Carolyane, J.A., Prakash, J., and Bhagya, S. (2002). Chemical, Nutritional, and functional properties of enzyme modified supplementary food formulations. *Bev. Food World*., 31.

Carpenter, K.J. (1960). The estimation of available lysine in animal protein foods. *Biochem.*, **77**, 604.

Carpenter, K.J., and Booth, V.H. (1973). Damage to lysine in food processing: its measurement and its significance. *Nutr. Abstr. Rev*., **43**, 424.

Cheryan, M. (1980). Phytic acid interaction in food systems. *CRC Crit. Rev. Food Sci. Nutr.*, **15**, 297.

Chiang, W.D., Shin, C.H., J., Chu, Y.H. (1999). Functional properties of soy protein hydrolysate produced from a continuous membrane reactor system. *Food Chem.*, **65**, 189.

Chichester, C.O. (1973). Nutrition in food processing. *World Rev. Nutr. Diet*., **16**, 318.

ePrint@CFTRI

Cho, Y.S., and Thompson, L.U. (1984). Precipitation behavior of extracted N, PA, and minerals in rapeseed flour modified by acylating agents. *J. Food Sci.*, **49**, 765.

Chobert, J.M., Briand, L., Gueguen, J., Popineau, Y., Larre, C., and Haertle, T. (1996). Recent advances in enzymatic modifications of food proteins for improving their functional properties. *Die Nahrung*, **40**, 177.

Chobert, J.M., Sitohy, M.Z., and Whitaker, J. (1988b). Solubility and emulsifying properties of caseins modified enzymatically by *Stphylococcus aureus* V8 protease. *J. Agric. Food Chem*., **36**, 220.

Chobert, J.M., Bertrand-Harb, C., and Nicolas, M.G. (1988a). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem*., **36**, 883.

Cigic, B., and Zelenik-Blatnik, M. (2004).Preparation and characterization of chicken egg white hydrolysate. *Acta Chem. Slov*., **51**, 177.

Clandinin, D.R., Renner, R., and Robblee, A.R. (1959). Rapeseed oil meal studies. 1.Effects of variety of rapeseed, growing environment and processing temperatures on the nutritive value and chemical composition of rapeseed oil meal. *Poultry Sci.*, **38**, 1367.

Cogan, U., Moshe, M., and Mokady, S. (1981). Debittering and nutritional upgradation of enzymic casein hydrolysates. *J. Sci. Food Agric*., **32**, 459.

Concon, J.M. (1975). Rapid and simple method for the determination of tryptophan in cereal grains. *Anal. Biochem*., **67**, 206.

Cosgrove, D.J. (1980). *Inositol phosphates:Their chemistry, biochemistry and physiology*. Elsevier Sci. Publ. Co., New York. NY.

Crenwelge, D., Dill, C.W., Tybor, P.T., and Landmann, W.A. (1974). A comparison of the emulsification capacities of some protein concentrates. *J. Food Sci*., **93**, 175.

Crisan, E.V., and Sands, A.(1978). Nutritional value of edible mushroom. In *Biology and Cultivation of Edible Mushrooms*. Chang, S.T., and Hayer W.A., (Eds.), PP: 137-168. Academic Press. New York.

ePrint@CFTRI

Croft, A. (1979). Determination of total glucosinolates in rapeseed meal by titration of enzymatically released acid. *J. Sci. Food Agric.,* **30**, 417.

Cubadda, R. (1994). Nutritional value of pasta. Effects of processing conditions. *Int. Food Beverage Technol*., **3**, 27.

Cunin, C., Handschin, S., Walther, P., and Escher, F. (1995). Structural changes of starch during cooking of durum wheat pasta. *Lebensm. Wiss. U. Techno*l., **28**, 323.

Darwicz, M., Dziuba, J., Caessens, P.W. JR. (2000). Effect of enzymatic hydrolysis on emulsifying and foaming properties of milk proteins. A review. *Pol. J. Food Nutr. Sci*., **9/50**, 3.

Daun, J.K., and Hougen, F.W. (1977). Identification of sulfur compounds in rapeseed oil. *J. Am. Oil Chem. Soc*., **54**, 351.

Daxenbichler, M.E., and VanEtten,C.H.(1977). Glucosinolates and derived products in cruciferous vegetables: gas liquid chromatographic determination of the aglucon derivatives from cabbage. *J. Assoc. Off. Anal. Chem*., **60**, 950.

Deeslie, W.D., and Cheryan, M. (1988). Functional properties of soy protein hydrolysates from a continuous ultrafiltration reactor. *J. Agric. Food Chem*., **36**, 28.

Deng, Q.Y., Barefoot, R.R., Diosady, L.L., Rubin, L.J., and Tzeng, Y.M. (1990). Lysinoalanine concentrations in rapeseed protein meals and isolates. *Can. Inst. Food Sci. Technol. J*., **23**, 140.

DeRham, O., and Jost, T. (1979). Phytate-protein interactions in soybean extracts and manufacture of low-phytate soy protein products. *J. Food Sci*., **44**, 596.

Deshpande, S.S., Cheryan, M., and Salunkhe, D.K. (1986). Tannin analysis of food products. *CRC Crit. Rev. Food Nutr*., **24**, 401.

Dev, D.K., and Mukherjee, K.D. (1986). Functional properties of rapeseed protein products with varying phytic acid content. *J. Agric. Food Chem*., **34**, 775.

ePrint@CFTRI

Dexter, J.E., Dronzek, B.L., and Matsuo, R.R. (1978). Scanning electron microscopy of cooked spaghetti. *Cereal Chem*., **55**, 23.

Dexter, J.E., Killborn, R.H., Morgan, B.C., and Matsuo, R.R. (1983). Grain research laboratory compression tester: Instrumental measurement of cooked spaghetti stickiness. *Cereal Chem*., **60**, 139.

Dhingra, S., and Kapoor, A.C. (1985). Nutritive value of mango seed kernel. *J. Sci. Food Agric.*, **36**, 752.

Dietrych, S.D., and Oleszek, W. (1999). Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum Moench*) grain. *J. Agric. Food Chem*., **47**, 4384.

Dijkstra, D.S., Linnemann, A.R., and Van Boekel, A.J.S. (2003). Toward sustainable production of protein rich foods: Appraisal of eight crops for Western Europe. Part II: Analysis of the technological aspects of production chain. *CRC Crit. Rev. Food Sci. Nutr.*, **43**, 481.

Diosady, L., Rubin, L., Tar, C., and Etkin, B.(1986). Air classification of rapeseed meal using the Tervel separator. *Can. J. Chem. Engin*., **64**, 768.

Diosady, L.L., Rubin, L.J., Tzeng, Y.M. (1989). Production of rapeseed protein materials. *U.S. Patent* 4, 889, 921.

Diosady, L.L., Tar, C.G., Rubin, L.J., and Naczk, M. (1987). Scale up of the production of glucosinolate-free canola meal. *Acta Aliment*., **16** , 167.

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.

Diosady, L.L., Xu, L., and Chen, B-K. (2003). Production of high-quality protein isolates from defatted meals of *Brassica*. *U.S. Patent* 20050042715.

Diosady, L.L., Xu, L., and Chen, B-K. (2005). Production of high-quality protein isolated from defatted meals of *Brassica* seeds. *U.S. Patent* 20050202154.

Donnelly, B.J. (1982). Teflon and non-Teflon lined dies: Effect on spaghetti quality. *J. Food Sci*., **47**, 1055.

ePrint@CFTRI

Douglass, J.S. and Mathews, R.H. (1982). Nutrient content of pasta products. *Cereal Foods World*, **27** ,558.

Downey, R.K., Bell, J.M. (1990). New developments in Canada research. In *Rapeseed and Canola: Chemistry, Nutrition and Processing Technology*. Shahidi, F., (Ed.), PP: 37-46. Norstrand Reinhold, New York.

Downey, R.K., Criag, B.M., and Youngs, C.G. (1969). Breeding rapeseed for oil and meal quality. *J. Am. Oil Chem. Soc*., **46**, 121.

Doyle, M.P., Applebaum, R.S., Brackett, R.E., and Marth, E.H. (1982). Physical, chemical and biological degradation of mycotoxin in foods and agricultural commodities. *J. Food Protect*., **45**, 964.

Duhan, A., Chauhan, B.M., Punia, D., and Kapoor, A.C. (1989). Phytic acid content of chickpea (*Cicer arientinum*) and blackgram (*Vigna mungol*): varietal differences and effect of domestic processing and cooking methods. *J. Sci. Food Agric*., **49**, 449.

Durkee, A., and Thivierge, P. (1975). Bound Phenolic acids in *Brassica* and *Sinapis* oilseeds. *J. Food Sci.,* **40**, 820.

Durkee, A.B. (1971). The nature of Tannin in rapeseed. *Phytochem*., **10**, 1583.

Durr, P. (1973). Enrichment of macaroni with milk protein. *Buhler Diag*., 57912.

Eapen, K.E., Tape, N.W., and Sims, R.P.A. (1968). New process for the production of better quality rapeseed oil and meal. I. Effect of heat treatments on enzyme destruction and color of rapeseed oil. *J. Am. Oil Chem. Soc*., **45**, 194.

Ekuland, A., Argen, G., and Langler, T.J. (1971). Rapeseed protein fractions. I. Preparation of detoxified lipid-protein concentrate from rapeseed (*Brassica napus* L.) by a water-ethanol extraction method. *J. Sci. Food Agric*., **22**, 650.

El-Nockrashy, A.S., Kiewitt, M., Mangold, H.K., and Mukherjee, K.D. (1975a). Nutritive value of rapeseed meals and rapeseed protein isolate. *Nutr. Metab*., **19,** 145.

ePrint@CFTRI

El-Nockrashy, A.S., Kiewitt, M., Mukherjee, K.D., and, Mangold, H.K. (1975b). New varieties of rapeseed as a source of protein. *Fette. Seifen. Anstrichm*., **77**,451.

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.

Erdman, J.W., Jr. (1979). Oil seed phytates: nutritional implications. *J. Am. Oil Chem. Soc.,* **56**, 736.

Ettlinger, M. G., and Dateo, G. P. (1961). Studies of mustard oil glucosides. U.S. Army Natick Laboratories, Natick, Mass. As cited by: Fenwick, G.R., Heaney, R.K., and Mullinn, W, J. (1983). *CRC Crit. Rev. Food Sci. Nutr.,* **18**, 123.

FAO (U.N.). (1975). *Protein requirements*. FAO Nutritional studies, Vol.16.

FAO/WHO. (1991). *Protein Quality Evaluation*. Food and Agricultural Organization of the United Nations, Rome, Italy, P: 66.

FAOSTAT, FAO, WHO (www.fao.org).

Fardet, A., Baldwin, P.M., Bertrand, D., Bouchet, B., Gallant, D.J., and Barry, J.L. (1998). Textural images analysis of pasta protein networks to determine influence of technological processes. *Cereal Chem*., **75**, 699.

FDA. (1993). Food labeling. Fed Req. **58** (3): 2101-2106.

Fenwick, G.R., and Curtis, R.F. (1980). Rapeseed meal and its use in poultry diets: a review. *Anim. Feed Sci. Technol*., **5**, 255.

Fenwick, G.R., Curl, C.L., Butler, E.J., Greenwood, N.M., and Pearson, A.W. (1984). Rapeseed meal and egg taints: effect of low glucosinolate *Brassica napus* meal, dehulled meal, hull and neomycin. *J. Sci. Food Agric*., **35**, 749.

Fenwick, G.R., Heaney, R.K., and Mulin, W.J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nutr*., **18**, 123.

ePrint@CFTRI

Fenwick, G.R., Spinks, E.A., Wilkinson, A.P., Heaney, R.K., and Legoy, M.A. (1986). Effect of processing on the anti-nutrient content of rapeseed. *J. Sci. Food Agric*., **37**, 735.

Fernandez, R., Elias, L.G., Edgar-Braham, J., Bressani, R. (1982). Trypsin inhibitor and haemagglutining in beans (*Phaseolus vulgaris*) and their relationship with the contents of tannins, and associated polyphenols. *J. Agric. Food Chem*., **36**, 734.

Finlayson, A.J. (1976). The seed protein contents of some Cruciferae. In *The Biology and Chemistry of Cruciferae*. Vaughan, J.G., Mc Leod, A.J., and Jones, B.M.G. (Eds.), Academic Press, London.

Finlayson, A.J., Bhatty, R.S., and Christ, C.M. (1969). Species and varietal differences in the proteins of rapeseed .*Can. J. Bot.,* **47**, 679.

Finlayson, A.J., Krzymanski, J., and. Downey, R.K. (1973). Comparison of chemical and agronomic characteristics of two *Brassica napus* cultivars, Bronowski and Target. *J. Amer. Oil Chem. Soc*., **50**, 407.

Finlayson, J. (1974). The amino acid composition of rapeseed hulls. *Can. J. Anim. Sci*., **54**, 495.

Finley, J.W., and Kohler, G.O. (1979). Processing conditions to inhibit lysinoalanine formation in alkaline treated proteins. *Cereal Chem*., **56**, 130.

Finnigan, T.J.A., and Lewis, M.J. (1985). Nitrogen extraction from defatted rapeseed with particular reference to United Kingdom commercial rapeseed. *J. Sci. Food Agric*., **36,** 520.

Food Agriculture Organization (FAO). (1968). *Amino acid content of foods and biological data on proteins*. Rome, UN: FAO.

Food Agriculture Organization/World Health organization (FAO/WHO). (1973). *Energy and protein Requirements*. Report of joint FAO/WHO Adhoc Expert Committee. WHO. Report series No.522; FAO Nutrition meeting report series No.2. Geneva: WHO, Rome, FAO.

Garten, V.A., and Weiss, D.E. (1957). The ion and electron-exchange properties of activated carbon in relation to its behavior as a catalyst and adsorbent. *Rev. Pure Applied Chem*., **7**, 69.

ePrint@CFTRI

Gill, T. A., and Tung, M.A. (1976). Rheological, chemical and microstructural studies of rapeseed protein dispersions. *Inst. Can. Sci. Technol. Aliment.*, **9,** 75.

Gillberg, L. (1978). Influence of electrolytes on the solubility of rapeseed protein isolate. *J. Food Sci.*, **43**, 1219.

Gillberg, L., and Tornell, B. (1976a). Preparation of rapeseed protein isolates: Dissolution and precipitation behavior of rapeseed protein. *J. Food Sci*., **41**, 1063.

Gillberg, L., and Tornell, B. (1976b). Preparation of rapeseed protein isolates. Precipitation of rapeseed protein in the presence of polyacids. *J. Food Sci*., **41**, 1070.

Girault, A. (1973). The study of some properties of rapeseed protein with a view to protein concentrate production. *J. Sci. Food Agric*., **24**, 509.

Glabe, E.F., Anderson, P.W., and Goldman, P.F. (1967). Macaroni made with non fat milk. *Cereal Sci. Today*., **12**, 510.

Goding, L.A., Bhatty, R.S., and Finlayson, A. J. (1970). The characterization of the 12S "globulin" from rapeseed and its glycoprotein component. *Can. J. Biochem*., **48**, 1096.

Goering, K.J. (1963). Obtaining nontoxic protein feed material from mustard seed, rapeseed and similar seeds. *U.S. Patent* 2, 987, 399.

Gunston, F.D. (2004). *Rapeseed and Canola oil: Production, Processing, Properties and uses*. Blackwell Publishing Ltd., CRC. Boca Raton, FL, USA.

Gururaj Rao, A., and Narasinga Rao, M.S. (1981). Comparative study of the high molecular weight protein fraction of mustard (*B. juncea*) and rapeseed (*B. campestris*). *Int. J. Pept. Protein Res.,* **18**, 154.

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.

Haber, T.A., Seyam, A.A., Banasik, O.J. (1978). Functional properties of some high protein products in pasta. *J. Agric. Food Chem*., **26**, 1191.

ePrint@CFTRI

Hanna, M.A., Satterlee, L.D., and Thayer, K.W. (1978). Sensory and selected textural properties of pasta fortified with plant proteins and whey. *J. Food Sci*., **43**, 231.

Helboe, P., Olsen, O., and Sørensen, H. (1980). Separation of glucosinolates by high performance liquid chromatography. *J. Chromatogr*., **197**,199.

Hermansson, A.M. (1975). Functional properties of proteins for foods: flow properties. *J. Texture Stud*., **5,** 425.

Hermansson, A.M., and Akesson, C. (1975). Functional properties of added proteins correlated with properties of meat systems: Effect of various parameters. *J. Food Sci*., **40**, 595.

Hermansson, A.M., Olsson, I., and Holmberg, B. (1974). Functional properties of proteins for foods. Modifications studies on rapeseed protein concentrates. *Lebensm. Wiss. U. Technol*., **4**, 201.

Hiron, S., Martens, R.W., and Murray, E.D. (2005). Canola protein isolate functionality II. *U.S. Patent* 20050064086.

How, J.S.L., and Morr, C.V. (1982). Removal of phenolic compounds from soy protein extracts using activated carbon. *J. Food Sci*., **47**, 933.

Hrckova, M., Rusnakova, M., and Zemanovic, J. (2002). Enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on functional properties of resulting protein hydrolysates. *Czech J. Food Sci*., **20**, 7.

Indian Standard Institution (ISI). (1993). Specification for Macaroni, Spaghetti and vermicelli. IS 1485.

Irvine, G. (1971). Durum wheat and pasta products In *Wheat: Chemistry and Technology*. Pomeranz, Y. (Ed.), 2nd Edn., Am. Assoc. Cereal Chem., St. Paul, MN. PP 777.

Irvine, G.N., Bradley, J.W., and Martin, G.C. (1961). A farinograph technique for macaroni dough. *Cereal Chem*., **38**, 153.

Ismond, M.A.H., and Welsh, W.D. (1992). Application of new methodology to canola protein isolate. *Food Chem*., **45**, 125.

ePrint@CFTRI

Jenkins, D.J., Wolever, T.M., Jenkins, A.L. (1988). Starchy foods and glycemic index. *Diabetes Care*, **11**, 149.

Jensen, S., Liu, Y., and Eggum, B. (1995). The influence of variations on seed size and hull content on composition and digestibility of rapeseed. In *proceeding 9th international rapeseed congress*, PP. 188-190. Cambridge.

Jensen, S.M., Olsen, H.S., and Sorensen, H. (1990). Aqueous enzymatic processing of rapeseed for production of high quality products. In *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*. PP: 331-343. Shahidi, F. (Ed.), AVI Book, New York.

Johnson, I.T. (2002). Glucosinolates: bioavailability and importance to health. *Int. J. Vit. Nutr. Res.*, **72**, 26.

Jones, J. (1979). Rapeseed protein concentrate preparation and evaluation. *J. Am. Oil Chem. Soc.*, **6**, 716.

Jones, J., and Holme, J. (1982). Oilseed processing. *Canadian patent* 1, 117, 134.

Jones, J., and Sibbald, I. (1979). The true metabolizable energy values for poultry of fractions of rapeseed *Brassica napus* CV Tower. *Poultry Sci*., **58**, 385.

Jones, J.D. (1976). Rapeseed in Canada. *Proceeding of the international symposium on rapeseed and mustard,* Mysore, India. PP: 6-12.

Jones, J.D. (1979). Rapeseed protein concentrate preparation and evaluation. *J. Am. Oil Chem. Soc.*, **56**, 716.

Jones, J.D., and Holme, J. (1979). Oilseed processing. *U.S. Patent* 4, 158, 656.

Jones, L.J., and Tung, M.A. (1983). Functional properties of modified oilseed protein concentrates and isolates. *Can. Inst. Food Sci. Technol. J*., **16**, 57.

Josefsson, E. (1970). Glucosinolate content and amino acid composition of rapeseed (*Brassica napus*) meal as affected by sulphor and nitrogen nutrition. *J. Sci. Food Agric*., **21,** 98.

ePrint@CFTRI

Josefsson, E. (1975). Effects of variation of heat treatment conditions on the nutritional value of low-glucosinolate rapeseed meal. *J. Sci. Food Agric*., **26**, 157.

Josefsson, E., and Munck, L. (1972). Influence of glucosinolates and a tentative high molecular detrimental factor on the nutritive value of rapeseed meal. *J. Sci. Food Agric*., **23**, 861.

Kakade, M.L., Rachis, J.J., McGhee, J.E., and Puski. G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chem*., **51**, 376.

Kanrharaj Urs, M., and Parpia, H.A.B. (1969). PAG Compendium, Vol. C2, Document 2.35/1 C, 1283.

Kantharaj Urs, M., and Kowsalya, S.M. (1976). Integrated processing of mustard seed for food and feed purposes. In *proceeding International symposium on rapeseed and mustard*. PP: 162-166. Mysore, India.

Karamac, M., Amarowicz, R., and Kostyra, H. (2002). Effect of temperature and enzyme/substrate ratio on hydrolysis of pea protein isolates by trypsin. *Czech J. Food Sci.*, **20**, 1.

Karcher, A., Melouk, H.A., and El-Rassi, Z. (1999). High performance liquid-phase separation of glycosides. III. Determination of total glucosinolates in cabbage and rapeseed by capillary electrophoresis via the enzymatically released glucose. *Anal. Biochem*., **267**, 92.

Keetels, C.J., A.M. Van Vliet, T., Jurgens, A., and Walstra, P. (1996). Effects of lipid surfactants on the structure and mechanics of concentrated starch gels and starch bread. *J. Cereal Sci*., **24**, 27.

Keshavarz, E., Cheung, R., Lui, R., and Nakai, S. (1977). Adaptation of the three stage extraction process to rapeseed meal for preparation of colorless protein extracts. *J. Can. Inst. Food Sci. Technol*., **10**, 73.

Khalil, M., Ragab, M., and Hassanien, F.R. (1985). Some functional properties of oilseed proteins. *Die Nahrung*., **29**, 275.

ePrint@CFTRI

Kim, C.H., Kim, H.S., Jung, Y.H., and Kang, Y.J. (1992a). Conditions for hydrolysis of rapeseed proteins by pronase. *J. Korean Soc. Food Nutr*., **21**, 513.

Kim, C.H., Kim, H.S., Lee, J.S., and Kang, Y.J. (1992b). Functionality changes of rapeseed protein upon proteolysis. *J. Korean Soc. Food Nutr*. , **21**, 519.

Kim, S.Y., Park, P.S.W., Rhee, K.C. (1990). Functional properties of proteolytic enzyme modified soy protein isolate. *J. Agric. Food Chem*., **38**, 651.

Kinsella, E.J., Domodaran, S., and German, B. (1985). Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. In *New Protein Foods*. Altschul, A., and Wilcks, H.L. (Eds.), Academic Press, LNC, New York, USA.

Kinsella, J.E. (1976). Functional properties of proteins in foods: A survey. *CRC Crit. Rev. Food Sci. Nutr*., **7**, 219.

Kirk, L.D., Mustakas, G. C., Grifin, E.L., Jr., and Booth, A.N. (1971). Crambe seed processing: decomposition of glucosinolates (thioglucosides) with chemical additives. *J. Am. Oil Chem. Soc*., **48**, 845.

Kishore Kumar Murthy, N. V., and Narasinga Rao, M.S. (1984).Acid denaturation of mustard 12S protein. *Int. J. Pept. Protein Res*., **23**, 94.

Klockeman, D.M., Toledo, R., and Sims, K.A. (1997). Isolation and characterization of defatted canola meal protein. *J. Agric. Food Chem*., **45**, 3867.

Knorr, D. (1977). Protein recovery from waste effluents of potato processing plants. *J. Food Technol*., **12**, 563.

Knorr, D. (1980). Protein recovery from food wastes: the influence of various methods of proteins coagulation upon yield, quality and properties of potato protein concentrates. *J. Food Sci*., **45**, 1183.

Knorr, D. (1982). Effect of recovery methods on the functionality of protein concentrates from food processing wastes. *J. Food Process. Eng*., **5**, 215.

ePrint@CFTRI

Knuckles, B.E. (1988). Effect of phytate and other myo-inositol phosphate esters on lipase activity. *J. Food Sci*., **53,** 250.

Knuckles, B.E., and Betschart, A.A. (1987). Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. *J. Food Sci*., **25**, 719.

Kohler, G.O., and Knuckles, B.E. (1977). Edible protein from leaves. *Food Technol*., **31**, 191.

Kozlowska, H., and Zadernowski, R. (1983). Production of protein preparates from rapeseed. In *Proceeding 6th International Rapeseed Congress.* Paris. PP: 160-163.

Kozlowska, H., Naczk, M., Shahidi, F., and Zadernowski, R. (1991). Phenolic acids and tannins in rapeseed and canola. In *canola and rapeseed: production, Chemistry, nutrition, and Processing Technology.* Shahidi. F. (Ed.). PP: 193-210. AVI Book: NewYork, NY.

Kozlowska, H., Sabir, M., Sosulski, F., and Cox Worth, E. (1975). Phenolic constituents of rapeseed flour. *Can. Inst. Food Sci. Technol. J*., **8**, 160.

Kozlowska, H., Sosulski, F.W., and Youngs, C.G. (1972). Extraction of glucosinolates from rapeseed. *Can. Inst. Food Sci. Technol. J*., **5**, 149.

Kracht, W., Danicke, S., Kluge, H., Keller, K., Matzke, W., Hennig, U., and Schumann, W. (2004). Effect of dehulling of rapeseed on feed value and nutrient digestibility of rape products in pigs. *Archi. Anim. Nutr*., **58**, 389.

Kracht, W., Jeroch, H., Daenicke, S., and Matzke, W. (1999). Effect of dehulling rapeseed on feed value of rapeseed meal and cake for poultry. In *10th international rapeseed congress*, Canberra, Australia.

Kroll, J., Mujaw, M., and Schnaak, W. (1991). Preparation of rapeseed proteins by extraction, ultrafiltration and diafiltration. *Food Sci. Technol*., **2**, 61.

Krygier, K., Sosulski, F., and Hogge, L. (1982). Free, esterified and insoluble phenolic acids.2. Composition of phenolic flour and hulls. *J. Agric. Food Chem*., **30**, 334.

ePrint@CFTRI

Kwee, W.H., Sidwell, V.D., Wiley, R.C., Hammerle, O.A. (1969). Quality and nutritive value of pasta made from rice, corn, soya and tapioca enriched with fish protein concentrate. *Cereal Chem.,* **46**, 78.

Labague, L., Grrat, G., Coustille, J.L., Viand, M.C., and Rollin, P. (1991). Identification of enzymatic degradation products from synthesized glucobrassicin by gas chromatography-mass spectrometry. *J. Chromatogra*., **586**,166.

Labuza, T.P., McNally, L., Gallagher, D., and Hurtado, F. (1972). Stability of intermediate moisture foods. *J. Food Sci*., **37**, 154.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680.

Lahl, W.J., and Braun, S.D. (1994). Enzymatic production of protein hydrolysates for food use. *Food Technol*., **48**, 68.

Lawhon, J.T., and Carter, C.M. (1971). Effect of processing method and pH of precipitation on the yields and functional properties of protein isolates from glandless cotton seed. *J. Food Sci*., **36**, 372.

Lein, K.A., and Schon, W.J., (1969). Quantitative determination of glucosinolates in *Brassica* half seeds. *Angew. Bot*., **43**, 87.

Leonardo, C., Giorgio, B., and Raffaeler, D. (1989). Method for black dyeing pasta foodstuffs and foodstuffs obtained by said method. *U.S. Patent* 4, 874, 619.

Leslie, A. J., Summers, J. D. (1975). Amino acid balance of rapeseed meal. *Poul. Sci*., **54**, 532.

Leung, J., Fenton, T., Mueller, M., and Clandinin, D. (1979). Condensed tannins of rapeseed meal. *J. Food Sci*., **44**, 1313.

Liesle, A., Summers, J., and Jones, J. (1973). Nutritive value of air-classified rapeseed fractions. *Can. J. Anim. Sci*., **53**, 153.

Lin, M.J., Humbert, E.S., and Sosulski, F.W. (1974). Certain functional properties of sunflower meal products. *J. Food Sci*., **39**, 368.

ePrint@CFTRI

Liu, R., Thompson, L.U., and Jones, J.D. (1982). Yield and nutritive value of rapeseed protein concentrate. *J. Food Sci*., **47**, 977.

Liu, Y-G, Jensen, S.K., and Eggum, B.O. (1995). The influence of seed size on digestibility and growth performance of broiler chickens fed full-fat rapeseed. *J. Sci. Food Agric*., **67**, 135.

Lo, M.T., and Hill, D.C. (1971a). Evaluation of protein concentrates prepared from rapeseed meal. *J. Sci. Food Agric*., **22**, 128.

Lo, M.T., and Hill, D.C. (1971b). Toxicity of a glucosinolate concentrate prepared from rapeseed meal. *Can. J. Anim. Sci*., **51**, 187.

Lo, M.T., and Hill, D.C. (1972). Composition of the aqueous extracts of rapeseed meals. *J. Sci. Food Agric*., **23**, 823.

Lonnerdal, B., and Janson, J. C. (1973). Studies on myrosinases. II. Purification and characterization of a myrosinase from rapeseed (*Brassica napus* L.). *Biochim. Biophys. Acta.*, **315**, 421.

Lonnerdal, B., and Janson, J.C. (1972). Studies on *Brassica* seed proteins.I. The low molecular weight proteins in rapeseed. Isolation and characterization. *Biochem. Biophys. Acta*., **278,** 175.

Lonnerdal, B., Gillberg, L., and Tornell, B. (1977). Preparation of rapeseed protein isolates: A study of rapeseed protein isolates by molecular sieve chromatography. *J. Food Sci*., **42**, 75.

Loomis, W., and Battaile, J. (1966). Plant phenolic compounds and isolation of plant enzymes. *Phytochem.*, **5**, 423.

Lorenz, K., Dilsaver, W., and Wolt, M. (1979). Fababean flour and protein concentrate in baked goods and in pasta products. *Baker's Dig*., **53**, 39.

Lu, S., Kim, H., Eskin, N., Latta, M., and Johnson, S. (1987). Changes in phytase activity and phytate during the germination of six canola cultivars. *J. Food Sci*., **52**, 173.

Maban, T.J. (1993). Color measurement of food. *Cereal Food World*., **38**, 21.

ePrint@CFTRI

Mackenzie, S.L. (1973). Cultivar differences in proteins of oriental mustard (*Brassica juncea* (L) Coss). *J. Am. Oil Chem. Soc*., **50**, 411.

Mackenzie, S.L., and. Blakely, J. A. (1972). Purification and characterization of seed globulins from *Brassica juncea*, *B. nigra* and *B. hitra*. *Can. J. Bot.,* **50**, 1825.

Madsen, J.S., Ahmt, T.O., Otte, J., Halkier, T., Qvist, K.B. (1997). Hydrolysis of β-lactoglobulin by four different proteinases monitored by capillary electrophoresis and high performance liquid chromatography. *Int. Dairy J*., **7,** 399.

Maga, J.A. (1982). Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *J. Agric. Food Chem.,* **30**, 1.

Maga, J.A. (1984). Lysinoalanine in foods. *J. Agric Food Chem*., **32**, 955.

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

Maheshwari, P.N., Stanley, D.W., Gray, J.I., and Van de Voort, F.R. (1979). An HPLC method for simultaneous quantitation of individual isothiocyanates and oxazolidinethione in myrosinase digests of rapeseed meal. *J. Am. Oil Chem. Soc*., **56**, 837.

Mahmoud, M.I. (1994). Physicochemical and functional properties of protein hydrolysates in nutritional products. *Food Technol*., **48**, 89.

Makkar, H.P.S. (1989). Protein precipitation methods for quantitation of tannins: a review. *J. Agric. Food Chem.,* 37, 1197.

Makkar, H.P.S., Singh, B., and Dawra, R.K. (1987). Tannin-nutrient interaction: a review. *Int. J. Anim. Sci*., **2**, 127.

Mandal, S., Kundu, P., Roy, B., and Mandal, R.K. (2002). Precursor of inactive 2S seed storage protein from the Indian mustard *Brassica juncea* is a novel trypsin inhibitor. *J. Biol. Chem*., **277**, 37161.

Mansour, E.H., Dworschak, E., Lugasi, A., Gaal, O., Barna, E, and Gergely, A. (1993). Effect of processing on the anti-nutritive factors and nutritive value of rapeseed products. *Food Chem*., **47**, 247.

ePrint@CFTRI

Manthey, F.A., and Hareland, G.A. (2001). Effects of break-roll differential on semolina and spaghetti quality. *Cereal Chem.,* **78**, 368.

Matill, K.F. (1971). The functional requirement of proteins for foods. *J. Am. Oil Chem. Soc*., **48**, 477.

Matsuo, R.R., and Irvine, G.N. (1970). Effect of gluten on the cooking quality of spaghetti. *Cereal Chem*., **47**, 173.

Matsuo, R.R., Bradley, J.W., and Irvine, G.N. (1972). Effect of protein content on the cooking quality of spaghetti. *Cereal Chem.,* **49**, 707.

Matsuo, R.R., Dexter, J.E., and Dronzek, B.L. (1978). Scanning electron microscopy study of spaghetti processing. *Cereal Chem*., **55**, 744.

Mawson, R., Heaney, R.K., Pisula, M., and Kozlowska, H. (1993). Rapeseed meal-glucosinolates and their anti-nutritional effects. Part I. Rapeseed production and chemistry of glucosinolates. *Die Nahrung,* **37**, 131.

Mayton, H.S. (1996). Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanates production in macerated leaf tissue. *Phytopathol*., **86**, 267.

McCormick, R.D. (1975). Improved texture, flavor and nutritional enhancement of pasta products. *Food Prod. Dev*., July-Aug, 11.

McCurdy, S.M. (1990). Effect of processing on functional properties of canola/rapeseed protein. *J. Am. Oil Chem. Soc.,* **67,** 281.

McFarlane, N., Shah, E., and McFarlane, M. (1976). Aqueous fractionation of rapeseed. *Tropical Sci*., **18**, 211.

McGhee, J.E., Kirk, L.D., and Mustakas, G.C. (1965). Methods for determining thioglucosides in *Crambe abyssinicia*. *J. Am. Oil Chem. Soc*., **42**, 889.

McGregor, D.I. (1983). Detoxification process for *Brassica juncea* seed. *U.K. Patent Application* 2, 113, 970.

McWatters, K.H., and Cherry, J.P. (1977). Emulsification, foaming and protein solubility properties of defatted soybean, peanut, field pea and pecan flours. *J. Food Sci.,* **42**, 1444.

ePrint@CFTRI

McWatters, K.H., and Cherry, J.P. (1983). Potential food uses of peanut seed proteins. In *Peanut Science and Technology*. Pattee, H.E., and Young, C.I., (Eds.), American Peanut Research Education Society. Yoakum, Texas.

Mieth, G., Bruckner, J, Kroll, J., and Phol, J. (1983b). Rapeseed: constituents and protein products. Part 2: Preparation and properties of protein-enriched products. *Die Nahrung*., **27**, 759.

Mieth, G., Schwenke, K.D., Raab, B., and Bruckner, J. (1983a). Rapeseeds constituents and protein products. Part 1. Composition and properties of proteins and glucosinolates. *Die Nahrung*., **27**, 675.

Mills, J.T., and Chong, J. (1977). Ultrastructure and mineral distribution in heat-damaged rapeseed. *Can. J. Plant Sci*., **57**, 21.

Mitaru, B., Blair, R., Bell, J., and Reichert, R. (1982). Tannin and Fibre contents of rapeseed and canola hulls. *Can. J. Anim. Sci*., **62**, 661.

Mithen, R.F., Dekker, M., Verker, R., Rabot, S., and Johnson I.T. (2000). The nutritional significance biosynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric*., **80**, 967.

Morad, M.M., El-Magoli, B., and Afifi, S.A. (1980). Macaroni supplemented with lupin and defatted soybean flours. *J. Food Sci*., **45**, 404.

Morris, E.R. (1986). Phytate and dietary mineral bioavailability. In *Phytic Acid: Chemistry and Applications.* Graf, E. (Ed.), PP: 57-76, Minneapolis, Pilatus Press.

Mothes, R., Schwenke, K.D., Zirwer, D., and Gast, K. (1990). Rapeseed protein polyanion interactions. Soluble complexes between the 2S protein fraction (napin) and phytic acid. *Die Nahrung*, **34**, 375.

Mueller, M., Ryl, E., Fenton, T., and Clandinin, D. (1978). Cultivar and growing location differences on sinapine content of rapeseed. *Can. J. Anim. Sc*i., **58**, 579.

Mukherjee, K.D., Afzalpurkar, A.B., and El-Nockrashy, A.S. (1976). Production of low glucosinolate rapeseed meal. *Fette. Seife. Anstrichm*., **78**, 306.

ePrint@CFTRI

Mullin, W.J. (1978). High-performance liquid chromatography of organic isothiocyanates and their methanol-isothiocyanate addition compounds. *J. Chromatogr.*, **155**, 198.

Murray, D. E., and Westdal, P. S. (2005). Enhanced oilseed protein recovery. *U.S. Patent* 20050042715.

Mustakas, G.C., Kirk, L.D., Solmas, V.E., and Griffin, E.L. (1965). Mustard seed processing: Improved methods of isolating the pungent factor and controlling protein quality. *J. Am. Oil Chem. Soc*., **42**, 33.

Naczk, M., Amarowicz, R., Sullivan, A., and Shahidi, F. (1998). Current research developments on polyphenolics of rapeseed/canola: A review. *Food Chem*., **62**,489.

Naczk, M., and Shahidi, F. (1989). The effect of methanol-ammonia-water treatment on the content of sinapine acid in *Brassica* seeds. *Food Chem*., **31**, 159.

Naczk, M., Ickle, D.O., Pink, D., and Shahidi, F. (1996). Protein precipitating capacity of crude canola tannins: Effect of pH, tannin and protein concentrations. *J. Agric. Food Chem*., **44**, 1444.

Naczk, M., Nichols, R., Pink, D., and Sosulski, F. (1994). Condensed tannins in canola hulls. *J. Agric. Food Chem*., **42**, 2196.

Naczk, M., Wanasundara, P., and Shahidi, F. (1992). Facile spectroscopic determination of sinapine acid in *Brassica* seeds. *J. Agric. Food Chem.,* **40**, 444.

Nagano, S., and Okamoto, S. (1976). Fractionation and characterization of globulin from rapeseed. *J. Japan Soc. Food Nutr.,* **29**, 433.

Nakai, S., Ho, L., and Tung, M.A. (1980). Solubilization of rapeseed, soy and sunflower protein isolates by surfactant and proteinase treatment. *Can. Inst. Food Sci. Technol. J.*, **13**, 14.

Narayana, K., and Narasinga Rao, M.S. (1982). Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour. *J. Food Sci*., **47**, 1534.

ePrint@CFTRI

Nielsen, M.A., Sumner, A.K., Whalley, L.L. (1980). Fortification of pasta with pea flour and air-classified pea protein concentrate. *Cereal Chem*., **57**, 203.

Nils, G.A., Johansson, C.G., Hallmer, H., and Siljestrom, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem*., **31**, 476.

Nishie, K., and Daxenbichler, M.E. (1980). Toxicity of glucosinolates related compounds (nitriles, R-goitrin, isothiocyanates) and vitamin K found in Cruciferae. *Food Cosmet. Toxicol*., **18**, 159.

Nwokolo, E.N., and Bragg, D.B. (1977). Influence of PA and crude fiber on the availability of minerals from four protein supplements in growing chicks. *Can. J. Anim. Sci.*, **57**, 475.

O'Dell, B.L. (1969). Effect of dietary components upon zing availability. *Am. J. Clin. Nutr.* , **22**, 1315.

O'Dell, B.L., and De Boland, A. (1976). Complexation of phytate with proteins and cations in corn germ and oilseed meal. *J. Agric. Food Chem*., **24**, 804.

O'Meara, G.M., Munro, P.A. (1985). Kinetics of the hydrolysis of lean meat protein by alcalase: Derivation of two alternative rate equations and their fit to experimental data. *Biotechnol. Bioeng*., **27**, 861.

Oatway, L., Vasanthan, T.V., and Helm, J.H. (2001). Phytic acid. *Food Rev. Int*., **17,** 419.

Ohlson, R. (1972). In *Rapeseed. Cultivation, Composition, Processing and utilization.* Appleqvist, L.A., and Ohlson, R. (Eds.), Elsevier, Amsterdam, P. 9.

Ohlson, R. (1976). Processing effects on oil quality. *J. Am. Oil Chem. Soc*., **53**, 299.

Ohlson, R., and Anjou, K. (1979). Rapeseed protein products. *J. Am. Oil Chem. Soc*., **56**, 431.

Ohlson, R., and Tear, J. (1974). *Proceedings of the 4th International Rapeseed Conference*, Giessen, Germany. P: 663.

ePrint@CFTRI

Ohtsuru, M., and Hata, T. (1979). The interaction of L-ascorbic acid with the active centre of myrosinase. *Biochem. Biophy. Acta*., **567,** 384.

Olsson, K., Theander, O., and Aman, P. (1976). Determination of total glucosinolate content in rapeseed and turnip rapeseed meals by gas liquid chromatography. *Swed. J. Agric. Res*., **6**, 225.

Ooraikul, B., Mei, H.A., Sarkar, S.K., and Jakson, H. (1980). Utilization of rapeseed meal in source production. *J. Food Sci*., **45**, 200.

Oser, B.L. (1951). Methods for the integrating essential amino acid content in the nutritional evaluation of protein. *J. Am. Dietetic Assoc*., **27,** 399.

Oser, B.L. (1959). An integrated essential amino acid index for predicting the biological value. In *Protein and Amino Acid Nutrition*, Albanese, A. A. (Ed.), PP: 281-295. Academic Press. New York.

PAG Compendium. (1975). *Protein-Calorie Advisory Group of the United Nation System.* Vol C., New York, USA.

Pagani, M.A., Gallant, D.J., Bouchet, B., and Resmini, P. (1986). Ultrastructure of cooked spaghetti. *Food Microstruct.*, **5**, 111.

Panyam, D., and Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends. Food Sci. Technol*., **7,** 120.

Paquet, A., and MA, C-Y. (1989). Racemization assessment in alkali treated dietary proteins using high-performance liquid chromatography. *Nutrition Res*., **9,** 1053.

Paulsen, T.M. (1961). A study of macaroni products containing soy flour. *Food Technol*., **15**, 118.

Pirie, N.W. (1975).*Food protein sources*. Cambridge University Press, Cambridge.

Pokorny, J., and Sefr, Z. (1964). Scientific papers from *Inst. Chem. Techn. Prague Food Techno*l., **18,** 239,: as cited in Gururaj Rao, A., Ph.D. Thesis, University of Mysore, Mysore, India. 1980.

ePrint@CFTRI

Pomeranz, Y., Carvajal, M.J., Shrogen, M.D., Hoseney, R.C., and Finney, K.F. (1970). Wheat germ in bread making. II. Improving bread making properties by physical and chemical methods. *Cereal Chem*., **47**, 429.

Prakash, V., and Narasinga Rao, M.S. (1986). Physicochemical properties of oilseed proteins. *CRC Cri. Rev. Biochem.*, **20,** 265.

Puski, G. (1975). Modification of functional properties of soy proteins by proteolytic enzyme treatment. *Cereal Chem*., **52**, 655.

Puttaraj, S., Bhagya, S., Murthy, K.N., and Singh, N. (1994). Effect of detoxification of castor seed (*Ricinus Communis*) protein isolate on its nutritional quality. *Plant Food Hum. Nutr*., **46**, 63.

Quinn, J.R., and Jones, J.D.(1976). Rapeseed protein: pH solubility and electrophoretic characteristics. *J. Inst. Can. Sci. Technol. Aliment*., **9,** 47.

Rachberger, Y., Mokady, S., and Cogan, U. (1979). The effect of aqueous leaching of glucosinolates on the nutritive quality of rapeseed meal. *J. Sci. Food Agric.*, **30**, 31.

Rai, M., Singh, H., and Hedge, D.M. (2002). *Oilseeds and oils: Research and development needs*. Indian Society of Oilseed Research. Hyderabad.

Rakesh, J., and Metz, A. (1973). Acid precipitated fish protein isolate exhibits good functional properties. *Food Prod. Dev*., **7**, 18.

Rayas-Duarte, P., Mock, C.M., and Satterlee, L.D. (1996). Quality of spaghetti containing buckwheat, amaranth and lupin flours. *Cereal Chem*., **73**, 381.

Rayner, C.J., and Fox, M. (1976). Amino acid digestibility studies of autoclaved rapeseed meals using an *in vitro* enzymatic procedure. *J. Sci. Food Agric*., **27**, 643.

Reddy, N.R., Sathe, S.K., and Salunkhe, D.K. (1982). Phytates in legumes and cereals. *Adv. Food Res*., **28**, 1.

Resmini, P., and Pagani, M.A. (1983). Ultrastructure studies of pasta: a review. *Food Microstruct*., **2**, 98.

ePrint@CFTRI

Reynolds, J.R., and Youngs, C.G. (1964). Effect of seed preparation on efficiency and oil quality in filtration extraction of rapeseed. *J. Am. Oil Chem. Soc*., **41**, 63.

Robbelen, G., Thies, W. (1980). Variation in rapeseed glucosinolates and breeding for improved meal quality. In *Brassica Crops and their Wild Allies. Biology and breeding* .Tsunoda, S., Hinata, K., and Gomez-Campo, C. (Eds.), Ch. 16, Japan Sci. Soc. Press, Tokyo, Japan.

Rubin, L.J., Diosady, L.L., and Tzeng, Y.M. (1990). Ultrafiltration in rapeseed processing. In *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*. Shahidi, F. (Ed.), PP: 307-330. AVI Book, NewYork.

Rubino, M.I., Arntfield, S.D., Nadon, C.A., and Bernatsky, A. (1996). Phenolic protein interaction in relation to the gelation properties of canola protein. *Food Res. Int*., **29**, 653.

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

Sands, D.C., McIntyre, J.L., and Walton, G.S. (1976). Use of activated charcoal for the removal of patulin from cider. *App. Environ. Micr*., **32**, 388.

Sarwar, G., and McDonough, F.E. (1990). Review of protein quality evaluation methods: evaluation of protein digestibility corrected amino acid score method for assessing protein quality of foods. J. *Assoc. Off. Anal. Chem*., **73,** 347.

Sarwar, G., Bell. J., Sharby, T., and Jones, J. (1981). Nutritional evaluation of meals and meal fractions derived from rape and mustard seed. *Can. J. Anim. Sci*., **61**, 719.

Sarwar, G., Blair, R., Friedman, M., Gumbmann, M.R., Hackler, L.R., Pellett, P.L., and Smith, T.K. (1984). Inter-and Intra-laboratory variability in rat growth assays for estimating protein quality of foods. *J. Assoc. Off. Anal. Chem*., **67**, 976.

Satterlee, L.D., Marshal, H.F., and Tennyson, J.M. (1979). Measuring proteins quality. *J. Am. Oil Chem. Soc*., **56**, 103.

ePrint@CFTRI

Schewenke, K.D., Kroll, J., Lange, R., Kujawa, M., Schnaak, W., and Steinert, A. (1990). Preparation of detoxified high functional rapeseed flours. *J. Sci. Food Agric*., **51**, 391.

Schewenke, K.D., Raab, B., Plietz, P., and Damaschun, G. (1983). The structure of the 12S globulin from rapeseed (*Brassica napus* L.). *Die Nahrung*, **27**, 165.

Schewenke, K.D., Raab, B., Uhlig, J., Tkocz, H., Behlke, J., Bottger, M., and Freimuth, U. (1973). Seed Proteins. IV. The isolation and characterization of albumins of sunflower seed and rapeseed. Die *Nahrung*, **17**, 791.

Schneider, F. (1979). Method of shelling oil- and protein-containing grains. *Canadian Patent* 1, 62, 118.

Schoppet, E.F., Sinnamon, H.Z., Talley, F.B., Panzer, C.C., and Aceto, N.C. (1976). Enrichment of pasta with cottage cheese whey proteins. *J. Food Sci*., **41**, 1297.

Seo, A., and Morr, C.V. (1985). Activated carbon and ion exchange treatments for removing phenolics and phytate from peanut protein products. *J. Food Sci*., **50**, 262.

Serranio, M., and Thompson, L.U. (1984). Removal of PA and protein-mineral-phytate interactions in rapeseed. *J. Agric. Food Chem*., **32**, 38.

Seyam, A.A., Banasik, O.J., Breen, M.D. (1983). Protein isolates from navy and pinto beans: Their uses in macaroni products. *J. Agric. Food Chem*., **31**, 499.

Shah, B.G., Jones, J.D., Mclaughlan, J.M., and Beare-Rogers, J.L. (1976). Beneficial effect of Zn supplementation in young rats fed protein concentrate from rapeseed or mustard. *Nutr. Rep. Int*., **13**, 1.

Shahidi, F. (1990). *Canola and Rapeseed: Production, Chemistry and Processing Technology.* AVI Book, Van Nostrand Reinhold Inc., New York, NY.

Shahidi, F., and Gabon, J.E. (1990). Fate of sinigrin in methanol/ammonia/water-hexane extraction of *B. juncea* mustard seed. *J. Food Sci*., **55**, 793.

ePrint@CFTRI

Shahidi, F., and Naczk, M. (1989). Effect of processing on the content of condensed tannins in rapeseed meals. A research note. *J. Food Sci*., **54**, 1082.

Shahidi, F., Naczk, M., Rubin L.J., and Diosady, L.L. (1988). A novel processing approach for rapeseed and mustard seed-removal of undesirable constituents by methanol-ammonia. *J. Food Protec*., **51**, 743.

Shofran, B.G., Purringto, S.T., Bredit, F., and Fleming, H. (1998). Antimicrobial properties of sinigrin and its hydrolysis products. *J. Food Sci.,* **63,** 621.

Siddiqui, I.R., and Wood, P.J. (1976). Structural investigation oxalate-soluble rapeseed (*Brassica campestris*) polysaccharides. *Carbohyd. Res*., **50**, 97.

Siddiqui, I.R., and Wood, P.J. (1977). Carbohydrates of rapeseed: a review. *J. Sci. Food Agric*., **28**, 530.

Siegel, A., Bhumiratana, A., and Lineback, D.R. (1975). Development, acceptability and nutritional evaluation of high-protein soy-supplemented rice noodles for Thai children. *Cereal Chem*., **52**, 801.

Sikorski, Z.E. (2001). *Chemical and functional properties of food proteins.* Technomic Publishing Company, Inc., Pennsylvania, USA.

Simard, C., Dupont, Y., and Boulet, M. (1977). Properietes physico-chimiquest et composition en acides amines des fractions de proteins de soya, de feverole, de colza et de feuilles de Luzerne. *Can. Inst. Food Sci. Technol. J.* **10**, 326.

Sims, R.P.A. (1971). Edible protein products from Cruciferae seed meals. *J. Am. Oil Chem. Soc*., **48**, 733.

Singh, H.G., Srivastava, A.N., and Singh, R.N. (1976). Current status of breeding Indian mustard (*Brassica Juncea* L. Czern and Coss) in Uttar Pradesh. In *Proceeding of international symposium on rapeseed and mustard*, PP: 42-47, Mysore, India.

Singh, U., Singh, B., Smith, O.D. (1991). Effect of varieties and processing methods on phytic acid and protein digestibility of groundnut (*Arachis Hypogaea* L.). *J. Food Sci. Technol*., **28**, 345.

ePrint@CFTRI

Siy, R.D., and Talbot, F.D.F. (1982). Preparation of low-phytate rapeseed protein by ultrafiltration. 1. The aqueous extraction of phytate from boiled rapeseed meals. *J. Am. Oil Chem. Soc*., **59**, 191.

Slinger, S.J. (1977). Improving the nutritional properties of rapeseed. *J. Am. Oil Chem. Soc.,* **54**, 94A.

Slominski, B.A., and Campbell, L.D. (1990). Non starch Polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. *J. Sci. Food Agric*., **53**, 175.

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.

Snedecor,G.W., Cochran, W.G. (1967). *Statistical Methods*. 6th Edn. Lowa State University Press, Ames, Lowa.

Soon Rhee, K., and Choon Rhee, K. (1981). Nutritional evaluation of proteins in oilseed products heated with sugars. *J. Food Sci*., **46**, 164.

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

Sosulski, F. W., and Sarwar, G. (1973). Amino acid composition of oilseed meals and protein isolates. *Can. Inst. Food Sci. Technol. J.*, **6**, 1.

Sosulski, F. W., and Zadernowski, R. (1981). Fractionation of rapeseed meal into flour and hull components. *J. Amer. Oil Chem. Soc*., **58**, 96.

Sosulski, F. W., Humbert, E.S., Bui, K., and Jones, J.D. (1976). Functional properties of rapeseed flours, concentrates and isolates. *J. Food Sci*., **41**, 1349.

Sosulski, F.W. (1962). The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem*., **39**, 344.

Sosulski, F.W., and Bakal, A. (1969). Isolated protein from rapeseed, flax and sunflower meals. *Can. Inst. Food Sci. Technol*., **2**, 28.

Sosulski, F.W., and Zadernowski, R. (1980). *Canadian Patent* 1, 089, 849.

ePrint@CFTRI

Sosulski, F.W., Soliman, F.S., and Bhatty, R.S. (1972). Diffusion extraction of glucosinolates from rapeseed. *Can. Inst. Food Sci. Technol. J*., **5**, 101.

Sovak, M. (2001). Grape extract, Resveratrol, and its analogs: a review. *J. Medicinal Food***, 4,** 93.

Spencer, G.F., and Daxenbichler, M.E. (1980). Gas chromatography-mass spectrometry of nitriles, isothiocyanates and oxazolidinethiones derives from cruciferous glucosinolates. *J. Sci. Food Agric*., **31**, 359.

Spinelli, J., and Koury, B.J. (1974). Preparation of functional fish protein concentrates and isolates. *U.S. Patent* 3, 826, 848.

Srivastava, V.K., and Hill, D.C. (1976). Effect of mild heat treatment on the nutritive value of low glucosinolate-low erucic acid rapeseed meals. *J. Sci. Food Agric.,* 27, 953.

Srivastava, V.K., Hill, D.C., and Slinger S.J. (1976). Comparison of some chemical characteristics of Indian and Canadian Brassica seeds. *Indian J. Nutr. Dietet.*, **13**, 336.

Stanley, D.W., Gill, T.A., Deman, J.M., and Tung, M.A. (1976). Microstructure of rapeseed. *Can. Inst. Food Sci. Technol. J*., **9**, 54.

Sternberg, M., Kim, C.Y., and Schwende, F.J. (1975). Lysinoalanine presence in food and ingredients. *Science*, **190**, 922.

Strolle, E.O., Cording, J., and Aceto, N.C. (1973). Recovering potato proteins coagulated by steam injection heating. *J. Agric. Food Chem*., **21**, 974.

Subba Rau, B.H., and Srinivasan, K.S. (1988). Enzymatic modification of groundnut flour (by papain/protease) and its effect on functional properties. *Lebensm. Wiss. U. Technol*., **21**, 126.

Swaisgood, H., and Catagani, L. (1991). Protein digestibility: In vitro methods of assessment. *Adv. Food Nutr. Res*., **35**, 185.

Swakais, M.P., and Pest, I.M. (1990). Determination of tryptophan in unhydrolysed food and feed stuff by acid ninhydrin method. *J. Agric. Food Chem*., **38**, 720.

ePrint@CFTRI

Tangendjaja, B., Buckle, K.A., and Wootton. M. (1980). Analysis of phytic acid by high-performance liquid chromatography. *J. Chromatogr*., **197**, 274.

Tape, N.W., Sabry, Z.I., Eapen, K.E. (1970). Production of rapeseed flour for human consumption. *Can. Inst. Food Sci. Technol. J*., **3**, 78.

TERI vision. (2001). World second largest grower of oilseeds wait for takers: what then, ail Indian oil seed quality? *Teri*, Issue No. **37**.

Theander, O., Aman, P., Miksche, G.E., and Yasuda, S. (1977). Carbohydrates, polyphenols and lignin in seed hulls of different colors of turnip rapeseed. *J. Agric. Food Chem*., **25**, 270.

Thompson, L.U. (1977). Co-precipitation of rapeseed and cheese whey protein using acid and heat treatment. *Can. Inst. Food Sci. Technol. J*., **10**, 43.

Thompson, L.U. (1987). Reduction of PA in protein isolates by acylation technique. *J. Am. Oil Chem. Soc*., **64**, 1712.

Thompson, L.U. (1990). *Canola and rapeseed: Production, Chemistry Nutrition and Processing Technology.* Shahidi, F., (Ed.), PP: 173-192. Avi Book, New York. NY.

Thompson, L.U. (1993). Potential health benefits and problems associated with anti-nutrients in foods. *Food Res. Int*., **26**, 131.

Thompson, L.U., Allum-Poon, P., and Procope, C. (1976). Isolation of rapeseed protein using sodium hexametaphosphate. *Can. Inst. Food Sci. Technol. J*., **9**, 15.

Thompson, L.U., and Cho, Y. (1984a). Effect of acylation upon extractability of N, minerals, PA in rapeseed flour and protein concentrate. *J. Food Sci*., **49**, 771.

Thompson, L.U., and Cho, Y. (1984b). Chemical composition and functional properties of acylated low-phytate rapeseed protein isolate. *J. Food Sci*., **49**, 1584.

Thompson, L.U., Liu, R.F.K. and Jones, J.D. (1982a). Functional properties and food applications of rapeseed protein concentrate. *J. Food Sci*., **47**, 1175.

ePrint@CFTRI

Thompson, L.U., Reyes, E.S., and Jones, J.D. (1982b). Modification of the sodium hexametaphosphate extraction-precipitation technique of rapeseed protein concentrate preparation. *J. Food Sci*., **47**, 982.

Torchinskii, Y.M., and Dixon, M.B.F. (1974). *Sulfhydryl and disulfide groups of proteins*. New York, Consultants Bureau.

Turgeon, S.L., Gauthier, S.F., Paquin, P. (1992). Emulsifying properties of whey peptide fractions as a function of pH and ionic strength. *J. Food Sci*., **57**, 601.

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.

Underhill, E.W., and Kirkland, D.F. (1971). Gas chromatography of trimethylsilyl derivatives of glucosinolates. *J. Chromatogr*., **57**, 47.

Vaccarino, C., Toscano, M.A., Tripodo, M.S. (1976). Detoxification of rapeseed by hydrolyzing treatment on whole seed. III. *Rivista Italiana delle sostanze Grasse*, **52**, 291 (*Food Sci. Technol. Abstract* 9, 5N268, 1977).

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.

VanEtten, C.H., Gagne, W.E., Robbins, D.J., Booth, A.N., Daxenbichler, M.E., and Wolff, I.A. (1969). Natural glucosinolates (thioglucosides) in foods and feeds. *J. Agric. Food Chem*., **17**, 483.

ePrint@CFTRI

VanMegen, W.H. (1983). Removal of glucosinolates from defatted rapeseed meal by extraction with aqueous ethanol. *Can. Inst. Food Sci. Technol. J*., **16**, 93.

Venkat Rao, S., Kowsalya, S. M., and Kurien, S. (1976). Nutritional aspects of the proteins of mustard. *Proceeding of the international symposium on rapeseed and mustard*, Mysore, India. PP: 115-120.

Vioque, J., Sanchez-Vioque, R., Clemente, A., Pedroche, J., Bautista, J., and Millan, F. (2000). Partially hydrolyzed rapeseed protein isolates with improved functional properties. *J. Am. Oil Chem. Soc*., **77,** 447.

Vioque, J., Sanchez-Vioque, R., Clemente, A., Pedroche, J., Bautista, J., and Millan, F. (1999). Production and characterization of an extensive rapeseed protein hydrolysate. *J. Am. Oil Chem. Soc*., **76**, 819.

Walsh, D.E., and Gilles, K.A. (1971). The influence of protein composition on spaghetti quality. *Cereal Chem*., **48**, 544.

Wang, J., and Kinsella, J.E. (1976). Functional properties of novel proteins: alfalfa leaf proteins. *J. Food Sci*., **41,** 286.

Wetter, L.R., and Youngs, C.G. (1976). A thiourea-UV assay for total glucosinolate content in rapeseed meals. *J. Am. Oil Chem. Soc*., **53**, 162.

Whitmore, B.B., and Naidu, A.S. (2000). Glucosinolates. In *Natural food antimicrobial systems*. Naidu, A.S., (Ed.), CRC Press. PP: 399-416.

Wolever, T.M. (1990). The glycemic index. *World Rev. Nutr. Diet*., **62**, 120.

Wolf, W.J. (1970). Scanning electron microscopy of soybean protein bodies. *J. Am. Oil Chem. Soc*., **47**, 107.

Wolf, W.J., and Baker, F.L. (1975). Scanning electron microscopy of soybeans, soy flours, protein concentrates and protein isolates. *Cereal Chem*., **52**, 387.

Wolf, W.J., and Cowan, J.C. (1975). *Soybeans as a Food Source*. 2nd Edn., CRC Press, Cleveland.

ePrint@CFTRI

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.

Wu, Y.V., and Inglett, G.E. (1974). Denaturation of plant proteins related to functionality and food applications: a review. *J. Food Sci*., **39**, 218.

Wu, Y.V., Hareland, G.A., Warner, K. (2001). Protein-enriched spaghetti fortified with corn gluten meal. *J. Agric. Food Chem*., **49**, 3906.

Xander, P.A., and Hoover, E.F. (1959). *U.S. Patent* 2, 879,264.

Xu, L., and Diosady, L.L. (1994). The production of Chinese rapeseed protein isolates by membrane processing. *J. Am. Oil Chem. Soc*., **71**, 935.

Xu, L., and Diosady, L.L. (2000). Interaction between canola protein and phenolic compounds in aqueous media. *Food Res. Int*., **33**, 725.

Xu, L., and Diosady, L.L. (2002). Removal of phenolic compounds in the production of high-quality canola protein isolate. *Food Res. Int*., **35**, 23.

Yiu, S.H., Altosaar, I., and Fulcher, R.G. (1983). The effects of commercial processing on the structure and microchemical organization of rapeseed. *Food Microstruct*., **2**, 165.

Yiu, S.H., Poon, H., Fulcher, R.G., and Altosaar, I. (1982). The microscopic structure and chemistry of rapeseed and its products. *Food Microstruct*., **1**, 135.

Youngs, C.G., and Perlin, A.S. (1967). Fe (II) catalyzed decomposition of sinigrin and related thioglucosides. *Can. J. Chem*., **45**, 1801.

Youngs, C.G., and Wetter, L.R. (1967). Micro-determination of the major individual isothiocyanates and oxazolidinethione in rapeseed. *J. Am. Oil Chem. Soc.*, **44**, 551.

Zakaria, F., and McFeeters, R.F. (1978). Improvement of the emulsification properties of soy protein by limited pepsin hydrolysis. *Lebensm. Wiss. U. Technol.*, **11**, 42.

ePrint@CFTRI

Zeb, A., Sattar, A., Shah, A.B., Bibi, N., Thinggaard, G., and Meulen, U.T. (2002). Effects of dehulling and dry heating on the nutritional value of rapeseed meal for broiler chicks. *Arch. Geflugelk*., **66**, 164.

Zhou, B., He, Yu, H-M, and Mukherjee, K.D. (1990). Proteins from double zero rapeseed. *J. Agric. Food Chem*., **38**, 690.

ePrint@CFTRI

1. Patent Filed: "A Process for the Preparation of Mustard Protein Isolate with Reduced Anti-Nutritional Factors". Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. Patent No. 480/DEL/04, 2004.

2. Evaluation of Mustard (*Brassica juncea*) Protein Isolate Prepared by Steam Injection Heating for Reduction of Anti-Nutritional Factors. Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. (2006). *Lebensm. Wiss. U. Technol.* (LWT) (in press).

3. A Method for Preparation of Protein Isolate with Low Content of Glucosinolates, Phenolics, and Phytates from Mustard. Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. 5th International Food Convention, IFCON 2003, CFTRI, Mysore, 5-8th December, 2003.

4. Chemical and Nutritional Evaluation of Mustard Protein with Reduced Anti-Nutritional Factors. Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. Nutrition Society of India XXXVI Annual Meet. University of Mysore. 5-6th November, 2004.

5. Studies on Preparation of Mustard Protein Isolate with Reduced Anti-Nutritional Constituents. Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. 16th Indian Convention of Food Scientists and Technologies (ICFOST-2004). 9-10th December Mysore, INDIA.

6. Effect of Incorporation of Mustard Protein Isolate on Quality Characteristics of Spaghetti. Sadeghi Mahoonak, A.R., Aalami, M., Leelavathi, K., and Bhagya Swamylingappa. 16th Indian Convention of Food Scientists and Technologies (ICFOST-2004), 9-10th December, Mysore, INDIA.

ePrint@CFTRI

7. Chemical, Functional and Nutritional Characteristics of an Enzyme Modified Protein Concentrate from Commercial Mustard Cake. Sadeghi Mahoonak, A.R., and Bhagya Swamylingappa. In "*colloquium on novel proteins in nutrition & health*", March 22nd 2005, CFTRI, Mysore, INDIA.

8. Upgradation of Commercial Mustard Cake as a Protein Source in Food/Feed Purposes. Sadeghi Mahoonak, A.R., and Bhagya Swamylingappa. XXXVII Annual Conference of the Nutrition Society of India, 18-19th November, 2005, National Institute of Nutrition, Hyderabad, INDIA.

9. Recovery of protein from Mustard Meal by Different Methods: Effect on Yield, Anti-Nutritional Constituents and Nitrogen Solubility. Sadeghi Mahoonak, A.R., Appu Rao, A.G., and Bhagya Swamylingappa. 17th Indian Convention of Food Scientists & Technologists (ICFOST 2005), 9-10th December, Bangalore, INDIA.

ePrint@CFTRI