PROTEIN QUALITY OF DEOILED RICE BRAN AND ITS USE IN FOOD PRODUCTS

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
BIOCHEMISTRY
(Minor Subject: Food Science and Technology)

By

Gurleen Singh Mann
(L-2010-BS-27-IM)

Department of Biochemistry
College of Basic Sciences and Humanities

LUDHIANA-141004

2016

CERTIFICATE I

This is to certify that the thesis entitled, **"Protein quality of deoiled rice bran and its use in food products"** submitted for the degree of **Integrated Master of Science (Hons.)** in the subject of **Biochemistry** (Minor subject: **Food Science and Technology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Gurleen Singh Mann (L-2010-BS-27-IM)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

(Dr. (Mrs.) Surekha Bhatia)
Major Advisor
Assistant Biochemist
Deptt. of Processing and Food Engineering
College of Agricultural Engineering and Technology
Punjab Agricultural University
Ludhiana-141004, India

CERTIFICATE – II

This is to certify that the thesis entitled, **"Protein quality of deoiled rice bran and its use in food products"**, submitted by **Gurleen Singh Mann (L-2010-BS-27-IM)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Biochemistry** (Minor subject: **Food Science and Technology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

_____
(Dr. (Mrs.) Surekha Bhatia)
Major Advisor

_____
(Dr. Balwant Singh)
External Examiner
Professor (Retd.)
Department of Molecular Biology & Biochemistry
Guru Nanak Dev University, Amritsar

_____
(Dr. (Mrs.) Bavita Asthir)
Head of the Department

_____
(Dr. (Mrs.) Neelam Grewal)
Dean Postgraduate Studies

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to GOD, the almighty, for his showers of blessings throughout my research work to complete the research successfully.

It is my privilege to express my sincere gratitude to my major advisor, Dr. Surekha Bhatia, Assistant Biochemist, Department of Processing and Food Engineering, for her continuous support to my M.Sc. study and related research. Her introspective guidance, supervision, constructive suggestions, healthy condemnation, assistance, patience, motivation, immense knowledge and support from the initial to the final level enabled me to develop an understanding of the research work.

I am very thankful to Dr. Bavita Asthir, Sr. Biochemist-Cum-Head, Department of Biochemistry, for providing encouragement and necessary facilities in carrying out the research work. I am also thankful for her valuable suggestions which incented me to widen my research from various perspectives.

I am especially grateful to Dr. Neerja Sharma, Sr. Biochemist (Dean PGS Nominee), Department of Biochemistry for her valuable suggestions and help provided during the study.

I lack words to express my cordial thanks to my advisory committee: Dr. Amarjit Kaur, Sr. Milling Technologist-cum-Head, Department of Food Science and Technology for her guidance, support and invaluable help to make this study a success.

Words are not adequate to express the depth of my gratitude towards my family: My grandparents, my father Davinder Singh Mann, my mother Daljit Kaur, my sister Ravleen Kaur Mann, my mama ji Bhupinder Singh, my brothers Rajbir Singh, Chandanpreet Singh, Jaspreet Singh and Jaskaranpal Singh and my masi ji for their love, affection, sacrifices, blessings and supporting me spiritually throughout the study and my life in general.

I thank my fellow labmates and friends; Ramandeep Kaur, Ishem Bhandohal, Maninder Kaur, Sukreeti Sharma, Seerat Bhinder, Ravneet Kaur, Ajit Kaur, Gurpreet Chaggar, Jagreet Singh, Gurkirat Singh, Swarnjeet Singh, Gurwinder Singh, Parsanpreet Singh, Harsimrandeep Singh, Jaspreet Singh, Sourav Singla, Tejinder Singh, Arashdeep Singh and Karanvir Singh for the stimulating discussions, ever-available cooperation and for their valuable support. A special thanks to my dear friend Ramandeep Kaur for her constant help and co-operation throughout the research work. I will remain in debt for her unawaring help, fruitful company and warm wishes. I will always be grateful to my best friends for their moral support and for all the fun we had in the last two years.

I acknowledge the wonderful support and help of all the staff members of the Department of Biochemistry for their help and co-operation while carrying out the experiments especially Mr. Harbans Singh and Mr. Dalbara Singh.

Gurleen Singh Mann

Title of the Thesis	**:**	Protein quality of deoiled rice bran and its use in food products
Name of the Student and Admission No.	**:**	Gurleen Singh Mann L-2010-BS-27-IM
Major Subject	**:**	Biochemistry
Minor Subject	**:**	Food Science and Technology
Name and Designation of Major Advisor	**:**	Dr. (Mrs.) Surekha Bhatia Assistant Biochemist
Degree to be Awarded	**:**	Integrated M.Sc. (Hons.) in Biochemistry
Year of award of Degree	**:**	2016
Total Pages in Thesis	**:**	49 + VITA
Name of the University	**:**	Punjab Agricultural University, Ludhiana-141004, Punjab, India

ABSTRACT

Deoiled rice bran protein (DRBP) was extracted using alkali extraction method and enzymatic extraction method. Optimization of protein extraction by alkali extraction method was done using box-behnken design of response surface methodology (RSM). Yield and protein content of protein concentrate were found to be affected by extraction conditions viz. bran/water ratio, pH and time. pH had most significant effect on yield of protein concentrate followed by time of extraction and bran/water of extraction solvent. Optimum process variables for extraction of protein concentrate from deoiled rice bran were 0.175 bran/water ratio, 9.5 pH of solution and 45 min of extraction time which gave 8.63% yield of protein concentrate having protein content of 83.75%. Adding 0, 700, 1400, 2100 units of amylase to 100g deoiled rice bran resulted in 7.08, 10.00, 12.87 and 13.50% yield of protein concentrate respectively with 57.29, 62.94, 65.86 and 58.46% protein respectively, whereas addition of 0.9, 1.8 and 2.7 units of enzyme protease resulted in 7.08, 11.80, 12.70 and 15.60% yield of protein concentrate with 71.33, 72.86 and 64.10% of protein content respectively. Albumin (37.23%), globulin (20.27%) and glutelin (46.82%) were found to be three major fractions of deoiled rice bran protein concentrate, while prolamin (1.18%) was a minor component. Highest antioxidative activity was detected in albumin fractions followed by glutelin, then globulin. Cookies with 10% and muffins with 15% level of incorporation of deoiled rice bran were most accepted, whereas 1% level of incorporation of deoiled rice bran protein concentrate in cookies was most liked by the panel of judges.

Keywords: antioxidant activity, cookies, deoiled rice bran, protein, muffins.

_____ _____

Signature of Major Advisor Signature of the Student

ਖੋਜ ਪ੍ਰਬੰਧ ਦਾ ਸਿਰਲੇਖ	:	ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਛਿਲਕੇ ਦੇ ਪ੍ਰੋਟੀਨ ਦੀ ਗੁਣਵਤਾ ਅਤੇ ਭੋਜਨ ਉਤਪਾਦਾਂ ਵਿੱਚ ਇਸਦੀ ਵਰਤੋਂ ਦਾ ਮੁਲਾਂਕਣ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਮ ਅਤੇ ਦਾਖਲਾ ਨੰ.	:	ਗੁਰਲੀਨ ਸਿੰਘ ਮਾਨ (ਐਲ-2010-ਬੀ.ਐਸ.-27-ਆਈ.ਐਮ.)
ਮੁੱਖ ਵਿਸ਼ਾ	:	ਜੈਵਿਕ-ਰਸਾਇਣ ਵਿਗਿਆਨ
ਨਿਮਨ ਵਿਸ਼ਾ	:	ਫੂਡ ਸਾਇੰਸ ਅਤੇ ਤਕਨੋਲੋਜੀ
ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਮ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ. ਸੁਰੇਖਾ ਭਾਟੀਆ ਸਹਾਇਕ ਜੈਵਿਕ-ਵਿਗਿਆਨੀ
ਡਿਗਰੀ	:	ਇੰਟੈਗਰੇਟਿਡ ਮਾਸਟਰ ਆਫ਼ ਸਾਇੰਸ (ਔਨਰਸ) ਜੈਵਿਕ-ਰਸਾਇਣ ਵਿਗਿਆਨ
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2016
ਖੋਜ ਪ੍ਰਬੰਧ ਦੇ ਕੁੱਲ ਪੰਨੇ	:	49 + ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ - 141004, ਪੰਜਾਬ, ਭਾਰਤ

ਸ਼ਾਰ-ਅੰਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ ਐਲਕਲੀ ਨਿਸ਼ਕਰਸ਼ਣ ਅਤੇ ਇੰਜ਼ਾਈਮੈਟਿਕ ਨਿਸ਼ਕਰਸ਼ਣ ਵਿਧੀ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਛਿਲਕੇ ਦੇ ਪ੍ਰੋਟੀਨ (DRBP) ਦਾ ਨਿਸ਼ਕਰਸ਼ਣ ਕੀਤਾ ਗਿਆ। ਰਿਸਪੌਂਸ ਸਰਫੇਟ ਮੈਥੇਡੋਲੋਜੀ (RSM) ਦੇ ਬਾੱਕਸ-ਬੈਹਕੈਂਟ ਡਿਜ਼ਾਈਨ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਐਲਕਲੀ ਨਿਸ਼ਕਰਸ਼ਣ ਵਿਧੀ ਰਾਹੀਂ ਨਿਸ਼ਕਰਸ਼ਤ ਕੀਤੇ ਪ੍ਰੋਟੀਨ ਦਾ ਅਨੁਕੂਲਨ ਕੀਤਾ ਗਿਆ। ਨਿਸ਼ਕਰਸ਼ਣ ਹਾਲਤਾਂ ਭਾਵ ਛਿਲਕਾ/ਪਾਣੀ ਅਨੁਪਾਤ, ਪੀ.ਐਚ. ਅਤੇ ਸਮੇਂ ਦਾ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦੇ ਝਾੜ ਅਤੇ ਪ੍ਰੋਟੀਨ ਮਿਕਦਾਰ ਉਪਰ ਪ੍ਰਭਾਵ ਵੇਖਣ ਨੂੰ ਮਿਲਿਆ। ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦੇ ਝਾੜ ਉਪਰ ਪੀ.ਐਚ. ਦਾ ਸਭ ਤੋਂ ਵਧੇਰੇ ਅਰਥਪੂਰਨ ਪ੍ਰਭਾਵ ਵੇਖਿਆ ਗਿਆ ਅਤੇ ਇਸ ਉਪਰੰਤ ਘੋਲਕਕ ਦੇ ਨਿਸ਼ਕਰਸ਼ਣ ਸਮੇਂ ਅਤੇ ਛਿਲਕਾ/ਪਾਣੀ ਅਨੁਪਾਤ ਦਾ ਪ੍ਰਭਾਵ ਦਰਜ ਕੀਤਾ ਗਿਆ। ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਛਿਲਕੇ ਤੋਂ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦੇ ਨਿਸ਼ਕਰਸ਼ਣ ਲਈ 0.175 ਛਿਲਕਾ/ਪਾਣੀ ਅਨੁਪਾਤ, ਮਿਸ਼ਰਣ ਦੀ 9.5 ਪੀ.ਐਚ. ਅਤੇ 45 ਮਿੰਟ ਨਿਸ਼ਕਰਸ਼ਣ ਸਮਾਂ ਸਭ ਤੋਂ ਅਨੁਕੂਲ ਪ੍ਰੋਸੈਸਿੰਗ ਵੈਰੀਏਬਲਾਂ ਸਨ ਜਿਹਨਾਂ ਨਾਲ 8.63% ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦਾ ਉਤਪਾਦਨ ਹੋਇਆ ਜਿਸ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਦੀ ਮਿਕਦਾਰ 83.75% ਸੀ। 100 ਗ੍ਰਾਮ ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਛਿਲਕੇ ਵਿੱਚ ਐਮਾਈਲੇਜ਼ ਦੇ 0, 700, 1400, 2100 ਯੂਨਿਟਾਂ ਦੀ ਵਰਤੋਂ ਕਰਨ ਨਾਲ ਕ੍ਰਮਵਾਰ 7.08, 10.00, 12.87 ਅਤੇ 13.50 ਪ੍ਰਤੀਸ਼ਤ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦਾ ਉਤਪਾਦਨ ਹੋਇਆ ਜਿਸ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਦੀ ਪ੍ਰਤੀਸ਼ਤਤਾ ਕ੍ਰਮਵਾਰ 57.29, 62.94, 65.86 ਅਤੇ 58.46% ਸੀ, ਜਦੋਂਕਿ ਇੰਜ਼ਾਈਮ ਪ੍ਰੋਟੀਏਜ਼ ਦੇ 0.9, 1.8 ਅਤੇ 2.7 ਯੂਨਿਟਾਂ ਦੀ ਵਰਤੋਂ ਨਾਲ ਕ੍ਰਮਵਾਰ 11.80, 12.70 ਅਤੇ 15.60 ਪ੍ਰਤੀਸ਼ਤ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦਾ ਉਤਪਾਦਨ ਹੋਇਆ ਜਿਸ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਦੀ ਪ੍ਰਤੀਸ਼ਤਤਾ ਕ੍ਰਮਵਾਰ 71.33, 72.86 ਅਤੇ 64.10% ਸੀ। ਐਲਬਿਉਮਿਨ (37.23%), ਗਲੋਬੁਲਿਨ (20.27%) ਅਤੇ ਗਲੂਟਾਲਿਨ (46.82%) ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਦੇ ਛਿਲਕੇ ਦੇ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦੇ ਤਿੰਨ ਪ੍ਰਮੁੱਖ ਅੰਸ਼ ਸਨ, ਜਦੋਂਕਿ ਪ੍ਰੋਲਾਮਿਨ (1.18%) ਲਘੂ ਅੰਸ਼ ਸੀ। ਐਲਬਿਉਮਿਨ ਅੰਸ਼ਾਂ ਵਿੱਚ ਐਂਟੀਆਕਸੀਡੇਟਿਵ ਗਤੀਵਿਧੀ ਸਭ ਤੋਂ ਵਧੇਰੇ ਦਰਜ ਕੀਤੀ ਗਈ ਅਤੇ ਇਸ ਉਪਰੰਤ ਗਲੂਟਾਲਿਨ ਅਤੇ ਗਲੋਬੁਲਿਨ ਵਿੱਚ ਐਂਟੀਆਕਸੀਡੇਟਿਵ ਗਤੀਵਿਧੀ ਸਭ ਤੋਂ ਵਧੇਰੇ ਪਾਈ ਗਈ।ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਛਿਲਕੇ ਦੇ 10% ਪੱਧਰ ਦੀ ਵਰਤੋਂ ਨਾਲ ਤਿਆਰ ਕੀਤੇ ਗਏ ਕੁਕਿਸ ਅਤੇ 15% ਪੱਧਰ ਨਾਲ ਤਿਆਰ ਕੀਤੇ ਗਏ ਮਫਿੰਨਸ ਸਭ ਤੋਂ ਵਧੇਰੇ ਸਵਿਕਾਰਤ ਸਨ, ਜਦੋਂਕਿ 1% ਚੌਲਾਂ ਦੇ ਤੇਲ ਨਿਸ਼ਕਰਸ਼ਤ ਪ੍ਰੋਟੀਨ ਕੰਨਸਨਟ੍ਰੇਟ ਦੇ 1% ਪੱਧਰ ਦੀ ਵਰਤੋਂ ਨਾਲ ਤਿਆਰ ਕੀਤੇ ਗਏ ਕੁਕਿਸ ਜੱਜਾਂ ਦੇ ਪੈਨਲ ਵੱਲੋਂ ਸਭ ਤੋਂ ਵਧੇਰੇ ਪਸੰਦ ਕੀਤੇ ਗਏ।

ਮੁੱਖ ਸ਼ਬਦ: ਐਂਟੀਆਕਸੀਡੈਨਟ ਗਤੀਵਿਧੀ, ਕੁਕਿਸ, ਪ੍ਰੋਟੀਨ, ਮਫਿੰਨਸ

ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa*) is one of the main cereal crops, as well as staple food for most of the world's population, especially Asian countries. The worldwide production area for rice is about 150 million hectares, while the annual production is about 661 million metric tons (USDA 2009). It is grown in more than 100 countries and there are around 18,000 varieties. The major rice producing continents are Asia, Africa and America. More than half of the production belongs to Asian countries such as China, India, Indonesia, Bangladesh, Veitnam and Thailand (Sereewatthanawut *et al* 2007). On average, per capita rice consumption is 56.9 kg per year. Consumption in the developing countries is around 68.5 kg per capita per year, and 12.8 kg per year for developed countries.

Rice as harvested from the field is called paddy. In the rice milling process, first the outermost layer (the hull) is removed to produce brown rice. This process is least damaging to the nutritional value of the rice and avoids the unnecessary loss of nutrients that occurs with the further processing to produce white milled rice (Kahlon *et al* 1990). Milling of paddy yields 70% of rice endosperm as the major product and by-products consisting of 20% rice husk, 8% rice bran and 2% rice germ. The brownish portion of rice which is taken out in fine grain form during dehusking and milling of paddy is called rice bran. It is the hard outer layer consisting of aleurone and pericarp. Rice bran contains many micronutrients like oryzanols, tocopherols, phytosterols, 20% oil, 15% protein, 50% carbohydrate (Prasad *et al* 2011).

Rice bran can serve as an animal feed as well as human food supplement and as a valuable source of edible oil. The quality of bran depends upon type of equipment used for milling of rice. Traditional milling of paddy by hand pounding or by small hullers yields bran heavily contaminated with husk, with high content of silica (>5%) and crude fibre. Therefore it had limited use even as an animal food. But now a days, in modern milling process the paddy is first dehusked by shellers to obtain husk and brown rice. Brown rice on further milling and polishing yields a good quality bran and polished rice separately with very less amount of husk content. The bran obtained by two step milling process can be used for health food (Prakash 1996). But it also has limited food application because of the rapid development of rancidity due to the activation of lipase in the bran upon milling that breaks down glycerides into fatty acids (Juliano 1985). Rice bran is usually not consumed as food also because of its high fiber content and possible hull contamination (Luh 1991).

The oil in rice bran is commercially extracted and used as high quality cooking oil. After removal of oil portion, the defatted rice bran is regarded as agricultural waste without further utilization, although it contains many useful substances. Deoiled rice bran contains

high level 12-15% of protein which has yet to be used to its full potential (Jongjareonrak *et al* 2015). Deoiled rice bran protein has been reported to be of high quality which has unique nutritional value and neutraceutical properties. Its protein is higher in lysine content than rice endosperm protein or any other cereal bran proteins (Juliano 1985). The protein efficiency ratio (PER) has been widely used as an indicator of protein nutritional quality. The PER values for rice bran protein concentrates range from 2.0 to 2.5, compared to 2.5 for casein. Protein digestibility of rice bran is greater than 90%. Rice bran is considered a good source of hypoallergenic proteins and may serve as a suitable ingredient for infant food formulations (Helm and Burks 1996). The nutritional and pharmaceutical potential of rice bran has been recognized. Recently rice bran protein (RBP) have been found to make a flavoring agent. Due to these multitudes of benefits, it is suitable to be used as functional ingredient in the food products.

At present, rice bran protein concentrates and isolates are not commercially available. This lack of availability could be due to the complex nature of proteins in rice bran and poor solubility of rice bran protein due to strong aggregation and extensive disulfide bond cross-linking (Hamada 1999). Also rice bran contains high phytate (1.7%) and fiber content (12%) which could bind with proteins, making the protein bodies very hard to separate from other components (Juliano 1985).

Several methods including alkaline method, enzymatic method and physical treatments have been applied to extract protein from rice bran. However, a large proportion of rice bran protein cannot be extracted by ordinary aqueous solvent because of its extensive disulfide bonds and aggregation. The most commonly used procedure for protein extraction from rice bran is an alkali hydrolysis method followed by acid precipitation. However, this method induces unfavorable chemical reactions that result in a loss of nutritive value and formation of toxic substances (DeGroot and Slump 1969). High concentration of solvent during alkaline extraction can solublize not only rice bran protein, it may change the molecular structure and nutritive value of the protein. Also the alkaline process of extraction can affect protein functional properties such as emulsification, film formation and whipping capacity due to protein denaturation (Kinsella 1981). Enzymatic methods have been attempted for rice bran protein production to avoid these unfavorable consequences. Another advantage of enzymatic hydrolysis method is the mild treatment. So it can be more easily controlled owing to inherent specificity of various proteases (Apinunjarupong *et al* 2009). Amylase, celluclast and viscozyme had been used to hydrolyze cellulose and hemi-cellulose to release bound protein to these components (Tang *et al* 2001). Proteases treatment could release more rice bran protein at high degree of hydrolysis ie.8%-9% (Hamada 2000).

The large quantity of deoiled rice bran is available at cheaper price, so it is necessary to utilize this un-trapped source of nutrients. An optimized method for isolation of deoiled rice bran protein concentrate is necessary to formulate the strategies for its application in food products. As a potential food ingredient, deoiled rice bran protein was subjected to various processing conditions leading to conformational and structural changes in the proteins (Adebiyi *et al* 2009). Information on the characteristics of protein concentrate extracted by different extraction methods is essential for their possible commercial application.

Recently people become more conscious about their diet plan. Rice bran has very high functional qualities with reduction of blood cholesterol levels and incidence of colon cancer. There have been different approaches to increase the dietary fibre content by using rice bran, in cookies, muffins, bread, crackers, pasteries and pancakes (Barber *et al* 1981).

Development of value-added products from deoiled rice bran proteins requires information on characteristics of protein extracted by good extraction method. Therefore keeping in view of the above facts the present investigation was undertaken with the following objectives:-

1) To optimize the extraction methods for deoiled rice bran proteins.

2) To isolate the deoiled rice bran protein fractions.

3) To utilize protein concentrates in wheat based food products.

CHAPTER – II

REVIEW OF LITERATURE

The literature related to present study on extraction and characterization of protein from deoiled rice bran has been reviewed under following subheadings.

2.1 Rice bran and deoiled rice bran

2.2 Extraction and characterization of protein from deoiled rice bran

2.2.1 Extraction by chemical method

2.2.2 Extraction by enzymatic method

2.2.3 Extraction by other methods

2.3 Functional properties of deoiled rice bran protein

2.4 Utilization of deoiled rice bran in food products

2.1 Rice bran and deoiled rice bran

Rice bran contains high amounts of beneficial antioxidants including tocopherols, tocotrienols and oryzanols. Lloyd *et al* (2000) investigated the changes in selected antioxidants in rice bran from both long and medium-grain rice during commercial milling and bran processing. Rice bran collected from various milling breaks of a commercial system had varying antioxidant levels. Bran collected after milling break 2 had the highest levels of tocopherol and tocotrienol. Oryzanol concentration was significantly higher in outer bran layers. Results indicated that the long-grain rice bran averaged 15% more antioxidants than the medium-grain rice bran.

Rice bran has been recognized as an excellent source of edible oil, protein, dietary fiber and allied micronutrients like minerals, vitamins and antioxidants. After stabilization and antinutritional appraisal, oil was extracted from bran samples (Iqbal *et al* 2005).

Rosniyana *et al* (2009) researched on the nutritional content and storage stability of stabilized rice bran. Study indicated that rice bran rich in fat, protein, mineral, vitamin, tocopherol and oryzanol. The nutritional composition of rice bran at 4% and 8% milling degree was stabilized by either autoclaving or parboiling process. The rice bran was autoclaved with commercial retort at 120^0C for 20 min. For the production of parboiled rice bran, the harvested paddy was soaked for 2h, steamed for 20 min, then dried and milled. Parboiled rice bran had significantly higher in nutritional contents than autoclaved rice bran. The value of fat, fiber, ash, most minerals and vitamins in parboiled bran were generally higher than treated by autoclave technique. The free fatty acid levels for both parboiled and autoclaved rice bran were below the 10% permissible level for 4 months and 6 months respectively for the product packed in oriented polypropylene packs, either vacuum or

without and stored in ambient temperature room conditions. These findings indicated that rice bran could be stored without risk of deterioration for long time prior being used for the production of many health related food products.

Jayadeep *et al* (2009) applied various physical processing methods like sieving, pin milling and air classification to upgrade the quality of bran and investigated its effect on particle size distribution, content of ash, protein, total dietary fiber, insoluble fiber, soluble fiber and oryzanol as well as total antioxidant activity in bran fractions. Sieve separation resulted in an increase in the content of protein and bio-functional components like soluble fiber and oryzanol as well as total antioxidant activity. Pin milling and sieving resulted in smaller particle size fraction without loss in the content of protein and other bio-functional components and antioxidant activity. Air classification of this material resulted in significant decrease in ash content with moderate increase in protein content and significant increase in the contents of oryzanol and soluble fiber and the total antioxidant activity.

2.2 Extraction and characterization of protein from deoiled rice bran

2.2.1 Extraction by chemical method

Connor *et al* (1976) extracted protein concentrates from full-fat rice bran with dilute sodium hydroxide at 24^0C, followed by separation of the fibrous residue, and heat or acid precipitation of the extracted protein. In an alternative procedure, the starch fraction was separated prior to protein precipitation. Protein concentrates containing 23-32% protein, 33-48% fat, and 15-23% starch were obtained in yields of 14-20%. Protein concentrates with the bulk of starch removed contained 33-38% protein and 49-55% fat. Fats were 86% unsaturated protein efficiency ratio and nitrogen digestibility of the concentrates were significantly greater than those of starting bran.

Protein content of 60-mesh, defatted rice (Oryza Sativa L.) meal was 10.6% on a dry weight basis. Fractionation of rice proteins yielded albumin, globulin, prolamin and glutelin in the proportions of 8:9.5:12.5:70, respectively. The ultraviolet absorption spectrum, amino acid composition, isoelectric points and subunit constitution of proteins were instinctly different for each fraction. Non-overlapping of isoelectric points and molecular weights of protein subunits suggested the absence of cross-contamination between various fractions. The respective chemical scores of albumin, globulin, prolamin and glutelin fractions were 47.5, 53.0, 23.0, and 46.7. leucine, lysine, sulfur-containing amino acids, and threonine were limiting amino acids of total proteins in rice with respective scores of 65.1, 66.3, 67.9 and 78.9. Estimates of biological values of protein fractions in human nutrition qualified albumins as superior and prolamins as inferior proteins. Two dimensional slab gel electrophoresis, with phenol-acetic acid-mercaptoethanol-urea (PAMU) system in the first dimension and sodium

dodecyl sulfate in the second dimension indicated that the mobility of protein in PAMU may depend on its molecular size (Padhye and Salunkhe, 1979).

Jiamyangyuen *et al* (2005) researched on extraction of rice bran protein concentrates and its application in bread. They extracted rice bran protein concentrate (RBPC) using alkaline extraction method with the objective to determine the optimal extracting conditions of RBPC. Central composite design was used for extraction. The response surface methodology was chosen to graphically express the relationship between pH and extraction time with the output variables of protein content and the percent yield of RBPC. It was found that optimal extracting conditions were pH 11 and 45 min which resulting in 69.16% protein content and 8.06% yield of RBPC. Extraction of rice bran protein concentrate (RBPC) from defatted rice bran was standardized using alkali extraction method.

Theerakulkait *et al* (2006) prepared rice bran protein extract (RBPE from laboratory defatted rice bran milled and sieved through 50 mesh screen) by alkali extraction. The extracting conditions that provided the maximum protein extractability were: extraction rice bran to water at ratio of 1:4 (w/v) at pH 9.5, agitating speed of 500 rpm for 45 min. the protein extractability was 44.4% of total protein in rice bran use for extraction. The results showed that protein solubility, emulsifying activity and emulsion stability index of RBPE were the highest at pH 9 compared to other pH ($p< 0.05$) with the values of 10.75%, 0.063(A 500nm) and 43.15 min, respectively, while the lowest at pH ($p<0.05$) with the values of 10.75, 0.063(A 500nm) and 18.58 min, respectively.

Adebiyi *et al* (2009) investigated on isolation and characterization of protein fractions from deoiled rice bran. Sequential extraction of rice bran protein (RBP) from defatted rice bran was conducted based on the differences in their solubility. Three extraction methods were investigated. Method 1 involved the isoelectric and acetone precipitation using water, 50 g kg^{-1} NaCl, 0.02 mol L^{-1} NaOH and 70% ethanol as extracting solvents for albumin (pH 4.1), globulin (pH 4.3), glutelin (pH 4.8) and prolamin, respectively. Method 2 adopted dialysis and sequential extraction was carried out with 20 g kg^{-1} NaCl, 70% ethanol, 0.1 mol L^{-1} acetic acid and 0.1 mol L^{-1} NaOH solution as extracting solvents. Method 3 combined dialysis, isoelectric and acetone precipitation for the extraction. Based on the yields and data obtained from sodium dodecyl sulphate polyacrylamide gel electrophoresis, size-exclusion chromatography and differential scanning calorimetry, method 3 was found to be best for the isolation of RBPs. Rice bran protein fraction (RBPF)—albumin, globulin, glutelin and prolamin were obtained in good yields. Denaturation temperature and enthalpy values of denaturation of RBPF vary. Highest phytate content was found in albumin and lowest in prolamin. The highest antioxidative and hemagglutinating activities were observed in albumin fraction.

Apinunjarupong *et al* (2009) extracted rice bran protein by using defatted rice bran and water at 1:6(w/w) and 6% of bromelain at pH 9.0, 50^0C, 500 rpm for 15 and 30 min. The degree of hydrolysis(DH) of rice bran protein extract(RBPE) was 19 and 36.5%, respectively and their nitrogen solubility was higher than the controls (without bromelain). Rice bran protein concentrate (RBPC) was prepared by spray drying. Emulsion activity of RBPC produced from 19% DH RBPE was increased while emulsion stability index was not significantly different from the control. Foam capacity and rehydration ability of RBPC were greater than the control.

Jongjareonrak *et al* (2015) optimized the procedure for protein extraction from deoiled Sangyod Phatthalung (Oryza sativa L.) rice bran using a central composite design (CCD) with three factors i.e sodium hydroxide concentration (0.05-0.2 M), extraction temperature (30-60°C) and extraction time (60-240 min) and determined the effect of these parameters on extraction yield and functional properties of rice bran protein. The optimum conditions obtained by using response surface methodology (RSM) were a sodium hydroxide concentration of 0.13 M at an extraction temperature of 49°C for 170 min where the protein extraction yield was 43.1%. The rice bran protein extract exhibited a maximum solubility at pH 10 (68.3%). The foaming activity, foaming stability, emulsifying activity index (EAI) and emulsion stability index (ESI) values of the rice bran protein at pH 10 were 113.4% v/v, 62.6 min, 0.170 (Abs500nm) and 37.1 min, respectively. The results indicated that protein can be extracted from deoiled rice bran with a high yield and can be used as a food ingredient or protein source.

2.2.2 Extraction by enzymatic method

Wang *et al* (1999) utilized phytase and xylanase for protein extraction from rice bran and found that the use of carbohydrases was beneficial in enhancing the yield of protein. Carbohydrases were thought to be involved in disintegration of cell wall tissue, facilitating protein extraction.

Hamada (2000) studied that large portions of rice bran protein could not be solubilized by mild solvents, but use of endoprotease increased protein recovery. Bran was treated with 2 commercial proteases to achieve 8% to 9% peptide bond hydrolysis. The exo-plus endprotease was preferred to just using endopeptidases as it allowed the production of protein hydrolysates with enhanced functional properties. Solubility and emulsification activity and stability of hydrolysates produced with the protease blends were greater than that produced with endoprotease alone. These high value hydrolysates, produced from rice bran, an underutilized rice milling co-product, were suitable for many processed foods, particularly those requiring potent solubility and emulsification at mildly acidic conditions.

Shih and Diagle (2000) used various enzymes to treat a protein-enriched rice flour for the production of rice protein isolates. The rice flour containing 49% protein was a by-product from the processing of brown rice for syrup production. The treatment sequence of α-amylase followed by glucoamylase was most effective, resulting in a product with 85% protein content. Treatment of product with a mixture of cellulase and xylanase, which raised the protein content in the insoluble fraction to 91%. Inorganic impurities, such as the metal manganese in the starting rice flour, were effectively removed. The recovered rice protein found practically intact and had relatively poor solubility and emulsification properties; however, these functional properties were improved substantially by adding xanthan gum as a functionality enhancing agent.

Hanmoungjai *et al* (2001) worked on enzymatic extraction of oil and protein from rice bran using a commercial protease (Alcalase) and evaluated the by response surface methodology. They found that the effect of enzyme concentration was most significant on oil and protein extraction yields, whereas incubation time and temperature had no significant effect. The maximal extraction yields of oil and protein were 79 and 68%, respectively. Further, the quality of oil recovered from the process in terms of free fatty acid, iodine value, and saponification value was comparable with solvent-extracted oil and commercial rice bran oil, but the peroxide value was higher.

The effectiveness of 3 carbohydrases for protein extraction from heat-stabilized defatted rice bran (HDRB) was evaluated. The optimal conditions of the enzyme required to extract the maximum amount of protein were investigated with quadratic Box-Draper D-optimal design and the data were analyzed with response surface methodology (Tang *et al* 2003). Amylase, viscozyme and celluclast extracted a maximum of 45.4, 12.1, and 28.5% protein, respectively. They showed that extracted protein ranged from 9.5 to 58.4% under conditions of water to bran ratio (5:1 to 20:1), a amylase (0 to 110000 units/10 g rice bran), temperature (35 to 55 °C), and time (1 to 8 h). The maximum protein extracted was 58.4% with water to bran ratio of 17:1, 87637 units amylase, and 50.9 °C. In another experiment, the maximum protein extracted was 53.2% at 10.3:1 water-to-bran ratio, 110000 units of amylase, and 55 °C of incubation temperature. These results suggested that impure food grade amylase containing protease was more effective than celluclast and viscozyme in protein extraction from HDRB.

Guan and Yao (2008) analyzed the enzymatic pretreatment of oat bran, using Viscozyme L to enhance protein extraction. Response surface methodology (RSM) was used to study the effects of pretreatment variables of Viscozyme L concentration (6–30 FBG), pH (3.0–5.0), incubation time (0.5–2.5 h) and temperature (35–55 °C) on protein extraction. The results indicated that protein extraction from oat bran was mainly affected by pH and

incubation temperature. Optimum conditions of enzymatic pretreatment were identified as Viscozyme L concentration 30 FBG/10 g of oat bran, pH 4.6, incubation time 2.8 h and temperature 44 °C where extracted protein was 56.2% which was significantly more than protein extracted by alkaline(pH 9.5) method (14.76%). They concluded that the enzymatic pretreatment method was more efficient in the oat bran protein extraction than was the alkaline method.

Xia *et al* (2012) used hydrothermal cooking (HTC) combined with amylase pretreatment (AP) to improve protein extraction from heat-stabilized rice bran. The physicochemical and emulsifying properties of rice bran protein isolate (RPI) were evaluated. Depending on HTC temperature (120 and 150 °C), HTC alone significantly increased extraction yield, while protein purity was decreased. In contrast, HTC combined with AP significantly improved both extraction yield and protein purity (about 45–50% and 72–74%, respectively). The AP avoided the co-precipitation of gelatinized starch during the acidic precipitation. Electrophoresis and size exclusion chromatography profiles indicated that HTC led to the dissociation of insoluble protein aggregates in rice bran, with subsequent increase of soluble aggregates in RPI, linked by non-covalent (e.g., hydrophobic interaction) and covalent bonds (disulfide bond). This result was evidenced by the increased disulfide bond contents and surface hydrophobicity of RPI. In addition, HTC-prepared RPI exhibited excellent emulsifying property.

2.2.3 Extraction by other methods

Hata *et al* (2007) treated defatted rice bran with subcritical water in the temperature range of 180- 280^{0}C for 5 min using 117 ml vessels to produce the extracts. The total sugar concentration of *ca*.0.3 g/L- extract was the highest for the extracts at 200^{0}C, and it significantly decreased at the higher temperatures. The protein concentrations and radical scavenging activity were higher at the higher temperatures. Extraction was also done at 200^{0}C and 260^{0}C for various times using the small vessels. The total sugar concentration decreased with the increasing extraction time, while the protein concentration and radical scavenging activity only slightly depended on the extraction time

Sereewatthanawut *et al* (2007) investigated the production of value-added protein and amino acids from deoiled rice bran by hydrolysis in subcritical water (SW) in the temperature range between 100 and 220^{0}C for 0–30 min. The results suggested that SW could effectively be used to hydrolyze deoiled rice bran to produce useful protein and amino acids. The amount of protein and amino acids produced are higher than those obtained by conventional alkali hydrolysis. The yields generally increased with increased temperature and hydrolysis time. However, thermal degradation of the product was observed when hydrolysis was carried out at higher temperature for extended period of time. The highest yield of protein and amino

acids were 219 ± 26 and 8.0 ± 1.6 mg/g of dry bran, and were obtained at 200^0C at hydrolysis time of 30 min. Moreover, the product obtained at 200^0 C after 30 min of hydrolysis exhibited high antioxidant activity and was shown to be suitable for use as culture medium for yeast growth.

Chiou *et al* (2012) obtained defatted rice bran extracts by subcritical treatment using aqueous acetone as extractant. Treatment with 40% (v/v) acetone at 230^0C for 5 min yielded an extract with the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (0.274 mmol of ascorbic acid/ g of bran), total carbohydrate (0.188g/g of bran), protein (0.512 g/g of bran), and total phenolic contents (88.2 mg of gallic acid/ g of bran). The effect of treatment temperature (70-230^0C) was investigated using 40% (v/v) acetone, and the extract under 230^0C treatment showed the highest levels of all the determinations described above. The extracts obtained with various concentrations of aqueous acetone were subjected to UV absorption spectra and HPLC analysis, and the results showed changes in composition and polarity.

Rahim *et al* (2015) extracted protein from raw rice bran by mixing full-fat unstabilized rice bran with water (5%, w/v) and autoclaved at 140 °C for 15, 30, 45 and 60 min. Soluble protein content and amino acid profile of the extracts were analyzed. 60 min autoclaving time produced extract with the best soluble protein content of 8.41 g/100g ±0.022 and the best amino acid profile with highest content of essential and conditional amino acids, 1.64 g/100g ±0.002 and 1.40 g/100g ±0.001, respectively. Sonication of the selected extract for 5 min before and after the autoclaving did not improved the protein extraction.

2.3 Functional properties of deoiled rice bran

Consumer attitude towards health foods is promising and the scope of functional foods is growing in the world markets.

Agboola and Mills (2005) worked on characterization of functional properties of australian rice protein isolates. Albumin, globulin, glutelin and prolamin fractions were isolated from an australian rice variety (cv. Langi) and characterized by yield, protein content and molecular weight profile using both capillary electrophoresis (SDS-CE) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The influence of pre-extraction enzymatic hydrolysis of starch and heating to 70 °C was also investigated, as was the extraction of the glutelin fraction without prior removal of the albumin and globulin fractions. Pre-extraction treatment affected mainly the albumin fraction, increasing dry matter yield but reducing protein content. SDS-CE was able to separate the protein fractions over a wider molecular weight range than SDS-PAGE, and the peaks from SDS-CE showed slightly higher molecular weight compared to equivalent bands from SDS-PAGE. This study has demonstrated that the method employed for isolating rice protein fractions, especially the pre-

extraction processing, affects their purity and protein profile by CE. The glutelin fraction extracted without prior removal of albumin and globulin fractions had different characteristics compared to those obtained by conventional extraction methods. Pre-extraction hydrolysis of starch did not significantly affect the emulsifying, foaming and gelling properties of extracted protein. Although rice glutelin had poor solubility, emulsifying and foaming properties in aqueous systems, it had good gelling properties which could be important for food applications.

The main aim of Parrado *et al* (2006) was the development of a new rice bran derivative and the description of its potential nutritional and health benefits. The chief functional feature of its chemical composition was that all the components are water soluble. The main component of rice bran enzymatic extract (RBEE) was proteins (38.1%) in the form of peptide and free amino acids – having a 6% content of sulfur amino acids. The second component was fat (30.0%), with oleic and linoleic acids as the major components, and 1.2 mg/g of c-oryzanol. Carbohydrates (14.2%) were comprised mainly of slowly absorbed carbohydrates.

Devi and Arumughan (2007) characterized the defatted rice bran (DRB) employing HPLC for identifying the major phytochemicals in DRB and to examine its commercial potential as a source of bioactive phytochemicals leading to value addition of DRB, otherwise used as cattle feed. Various solvent extracts showed the presence of oryzanols, tocols, and ferulic acid. Methanol was the most effective extractant under the optimized conditions of a material solvent ratio of 1:15 (wt./vol.) and a time of extraction of 10 h. The yields of total phenols, oryzanols and ferulic acid from DRB with methanol were 2204, 316, and 233 ppm, respectively. Enrichment of antioxidants in the crude methanolic extract (CME) was achieved by sequential extraction and fractionation, resulting in three enriched fractions-first was acetone extract (AE), second acetone extract-lipophilic fraction (AE-LP) and third acetone extract-polar fraction (AE-PP). While AE-LP was enriched in oryzanols and tocols by about 65 times, AE-PP was enriched in ferulic acid by 70 times as compared to their contents in DRB. Their study concluded that DRB could be a good commercial source of oryzanols, ferulic acid and other polyphenols and they can be separated and concentrated through simple solvent extraction and fractionation process as demonstrated here. The extracts and fractions enriched with oryzanols, tocols and ferulic acid with proven biological effects could be used as substitute for synthetic antioxidants for food products and as nutraceutical and cosmaceutical ingredients.

Commercial pea protein isolate was separated into 4 fraction first one was water-soluble (WS), second was salt-soluble (SS), third alkaline-soluble (AS) and fourth one was in ethanol-soluble (ES) fractions. AS fraction was the most abundant, constituting about 87% of

the proteins in PPI followed by WS, SS and ES fractions in decreasing order. ES fraction consistently formed emulsions with a narrow range of smaller oil droplet sizes (0.6–19 lm) at pH 4.0, 7.0 or 9.0 compared to a wider range of sizes for emulsions stabilized by WS, SS and AS fractions. Emulsions formed with ES fraction were also the most stable ($p < 0.05$) over the 3 h test period at all the pH values used in this work. The WS fraction had significantly highest ($p < 0.05$) protein solubility and foaming capacity at all the pH values when compared to solubility of PPI, SS, and ES. Except for AS and ES fractions, foaming capacities of the protein fractions were higher at pH 9.0 than at pH 4.0 or 7.0. Foam stability of PPI and SS fraction increased from pH 4.0 to 9.0, which may be due to increased charge density that prevented rapid coalescence of the air bubbles. The increase in charge density could have stabilized the foams by increasing electrostatic repulsions which reduced the rate of coalescence of foam particles (Adebeyi and Aluko 2011).

Zhang *et al* (2012) found that the rice bran proteins had molecular size between 0.1 and over 97.4 kDa; with maximum solubilities of 72.5% and 84.565 at pH 11; maximum emulsifying capacity of 0.149 and 0.634; maximum emulsion stability of 24.26 and 25.96 min; maximum foam capacity of 98% and 115%; maximum foam stabilities of 30.6 and 26.9 ml at 30 min; water absorption of 3.71 and 4.4 g/g and oil absorption of 4.24 and 5.13 g/g.

Several polyphenolic compounds have been recognised in the ethyl acetate extract of rice bran such as caffeic acid, ferulic acid, methoxycinnamic acid and vanillic acid which have been reported to inhibit the breast and colon cancer cells. Due to its anti-oxidative properties, the rice bran has the potential to be used as an additive to improve the storage stability in food products. RBP have been found to be high quality and application in food and pharmaceutical industry. Rice bran's unique properties, hypoallegenicity and anti-cancer effects make it a superior cereal protein with wide range of possible applications (Esa *et al* 2013).

2.4 Utilization of deoiled rice bran in food products

Yadav *et al* (2011) found that the protein content of biscuits increased significantly from 7.3 % in control biscuits to 15.4 % in the 15 % RBPC supplemented biscuits with their fracture strength also significantly higher than the control biscuits ($p<0.05$). Replacement of refined wheat flour up to 10 % RBPC produced protein-enriched biscuits with desirable overall acceptability. Rice bran protein concentrate can be beneficially utilized to formulate protein enriched biscuits with enhanced nutritional value especially for malnourished or undernourished people.

Jiamyangyuen *et al* (2005) found that when incorporating 1-5% of RBPC in a bread recipe, the weight loss and microbial counts of breads were decreased compared to those of control bread. The higher protein content and fiber in bread was corresponding to the amount

of RBPC added. Therefore, adding RBPC can significantly increase protein and fiber in bread. Addition of more than of RBPC decreased the sensory evaluation liking scores of color, taste, odor, texture and overall liking.

Sharif *et al* (2009) used defatted rice bran as supplement in preparation of fiber and mineral enriched cookies. Microwave stabilized defatted rice bran was supplemented in commercial straight grade wheat flour at 10, 20, 30, 40 and 50% supplementation level to prepare fiber and mineral enriched cookies. Cookies were analyzed for physical analysis, dietary fiber, mineral content (Na, K, Ca and Mg) and sensory attributes to find out the most suitable compositions for commercialization. Overall, rice bran supplementation improved dietary fiber content and mineral profile of the cookies. On the basis of physical analysis and sensory attributes, they concluded that defatted rice bran can be substituted upto 10 to 20% in wheat flour to prepare rice bran supplemented cookies without adversely affecting quality attributes.

CHAPTER III

MATERIAL AND METHODS

This study was conducted in the laboratory of Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana. The materials and methods employed in the present investigation have been described under following headings:

3.1 Procurement of deoiled rice bran

3.2 Chemicals

3.3 Proximate analysis of deoiled rice bran

3.4 Extraction of protein from deoiled rice bran

3.4.1 Extraction by using chemical method

3.4.2 Extraction by using enzymatic method

3.5 Estimation of extracted protein

3.5.1 Yield of protein

3.5.2 Estimation of protein

3.6 Optimization of protein extraction using Response surface methodology

3.6.1 Experimental design

3.6.2 Optimization of process parameters

3.7 Isolation and characterization of protein fractions

3.7.1 Isolation of protein fractions

3.7.2 Determination of anti-oxidative activity of protein fractions

3.7.3 SDS-PAGE of protein fractions

3.8 Preparation of cookies, muffins and their analyses

3.8.1 Preparation of cookies and muffins

3.8.2 Proximate analysis of cookies

3.8.3 Sensory analysis

3.8.4 Spread ratio of cookies

3.8.5 Specific volume of muffins

3.1 Procurement of deoiled rice bran

The present investigation was carried out on deoiled rice bran procured from solvent extraction plant located at Dhuri, Punjab.

3.2 Chemicals

All chemicals used in present study were of analytical grade.

3.3 Proximate analysis of deoiled rice bran

3.3.1 Moisture content (AOAC 2000)

The moisture content in the deoiled rice bran samples were determined by the hot air oven single stage method. Five grams of each sample was kept in an oven at 130°C for one

hour in weighed dish with cover. After one hour the sample dishes were kept in a dessicator to bring the sample to room temperature and weighed. Deoiled rice bran was reported as total solids and loss in weight as moisture. The formula used to calculate the moisture content was:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)}}{\text{Wt. of sample (g)}} \text{ x } 100$$

3.3.2 Crude protein (AOAC 2000)

a) Reagents:

i) **40% NaOH:** 40 g NaOH flakes added to distilled water to make volume 100ml.

ii) **Indicator:** 0.1 g methyl red and 0.5 gm bromocresol green were dissolved in 100 ml of 95% ethanol.

iii) **4% Boric acid solution:** 4 g boric acid was dissolved in distilled water to make volume 100 ml.

iv) **0.01N HCl:** 0.83 ml HCl dissolved in distilled water to make volume 1000 ml

b) Procedure:

Protein content was estimated by using Micro-Kjeldhal method. One gram of sample was weighed and added to the digestion flask. Then, 3-5 grams of digestion mixture (copper sulphate: potassium sulphate :: 1:9) was added to the flask and it was digested with 25 ml of concentrated sulphuric acid. The sample was digested till the solution became clear bluish green and all the nitrogen present was converted to ammonium sulphate. The solution was then cooled and volume of the digested sample was made to 250 ml with distilled water. To 50 ml of digested sample, 40 % sodium hydroxide solution was added to neutralize the acid and create a strong alkaline pH and it was immediately connected to a two-way condenser having 250 ml flask containing 25 ml of boric acid solution (4 %) with a mixed indicator at the other end. Distillation was carried out till the distillate became almost double its volume. Then, the distillate was titrated with 0.01 N hydrochloric acid to grey end point. Blank was also run along with the sample. Nitrogen (%) calculated was then multiplied with a factor of 6.25 to obtain % crude protein. Crude protein was calculated using the formula:

$$\text{\% Nitrogen} = \frac{\text{Volume of 0.01 N HCl used} \times 0.0014 \times \text{diluted factor}}{\text{Wt. of sample}} \times 100$$

$$\text{\% crude protein} = \text{\% Nitrogen} \times 6.25$$

3.3.3 Crude fat (AOAC 2000)

For the estimation of crude fat content Soxhlet method was used. Two grams of moisture free sample was weighed and transferred to thimble. Thimble with porosity was used to permit rapid passage of ether. Petroleum ether (40°- 60°C) was taken in the flasks

(200 ml) and apparatus was attached to condenser. The condenser was attached to tap for cold water circulation. Extraction was carried out for 8 hours. After extraction petroleum ether was evaporated and dried residue was cooled and weighed. The fat content of the samples were calculated as:

$$\text{Crude fat (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$

3.3.4 Ash (AOAC 2000)

Weighed (5 g) sample was first incinerated on hot plate until there were no more fumes. It was kept in a crucible to place in muffle furnance at 550°C for 5 hours, weighed and results were expressed in per cent.

$$\text{Ash (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample (g)}} \times 100$$

3.3.5 Crude fibre (AOAC 2000)

a) Reagents:

i) **1.25 % H_2SO_4 solution:** 1.3ml of conc. H_2SO_4 added to distilled water to make volume 100 ml

ii) **1.25% NaOH solution:** 1.25 g NaOH added to distilled water to make volume 100 ml

b) Procedure:

Crude fiber is defined as loss on ignition of dried residue remaining after digestion of sample with 1.25 % sulphuric acid and 1.25 % sodium hydroxide solution under specific conditions. Five grams of moisture and fat free sample was weighed and 200 ml of 1.25 % sulphuric acid was added to it. This solution was then boiled for 30 minutes and then filtered through Buchner funnel and filteration apparatus. The residue left behind was washed with water till it was acid free and residue was transferred to a beaker. Then, 200 ml of 1.25 % sodium hydroxide solution was added and again boiled for 30 minutes. The residues were again filtered and washed with water. Residues were then transferred to the pre-weighed crucible and dried to a constant weight at 100ºC in a hot air oven. This residue was then ashed in muffle furnace and loss in weight was recorded. Crude fiber was calculated using the formula:

$$\text{Crude fiber (\%)} = \frac{\text{(Weight of Residue – Weight of ash after ignition)}}{\text{Weight of sample}} \times 100$$

3.4 Extraction of protein from deoiled rice bran

3. 4.1 Extraction by using chemical method (Jiamyangyuen *et al* 2005)

Defatted rice bran sample and distilled deionized water (1:4) was pH adjusted to 9.5 and stirred 30 min at room temperature (RT). The slurry was centrifuged at 5000 g for 30 min

(RT). The pH of supernatant was adjusted to 4.5 and centrifuged again at 5000 g for 30 min. Precipitate was washed using water (pH 4.5). The residue was suspended in distilled deionized water (pH 7.0) and freeze dried overnight. The final product, which is called rice bran protein concentrate (RBPC), was then freeze dried and stored.

3.4.2 Extraction by using enzymatic method (Tang *et al* 2003)

To each 10 g of rice bran sample, 100 ml of deionized water was added and stirred to obtain a homogeneous slurry. The slurries were adjusted to: (1) pH 6.25 with 1.0 N HCl and 0/700/1400/2100 units of amylase was added; (2) For protease 0/0.9/1.8/2.7 units were added at pH 8.0.The slurries containing enzymes were mixed and those treated with amylase and viscozyme were incubated at 45 ℃ and shaken at 200 rpm in the shaker for 3.5 h. At the end of incubation, the slurries were centrifuged for 30 min at 1100 × *g* in a centrifuge. The pH of supernatant was adjusted to 4.5 and centrifuged again at 5000 g for 30 min. Precipitate was washed using water (pH 4.5). The residue was suspended in distilled deionized water (pH 7.0) and freeze dried overnight. The final product, which is called rice bran protein concentrate (RBPC), was then freeze dried and stored.

3.5 Estimation of extracted protein

3.5.1 Yield of protein

Percentage yield of protein was calculated of all the deoiled rice bran samples extracted under different treatment conditions.

Yield of protein was calculated by the following formula:

$$\text{Percentage yield of protein} = \frac{\text{RBPC obtained (g)}}{\text{Total amount of deoiled rice bran (g)}} \times 100$$

3.5.2 Estimation of proteins (Lowry *et al* 1951)

a) Reagents

i) Reagent A: 2% Na_2CO_3 was dissolved in 0.1 N NaOH.

ii) Reagent B: 0.5% $CuSO_4.5H_2O$ was dissolved in solution of sodium potassium tartarate.

iii) Alkaline copper tartarate: It was freshly prepared by mixing reagents 1 and 2 in ratio of 50:1 (v/v).

iv) Folin's reagent: Folin ciocalteau's phenol reagent was diluted with water in 1:1 ratio just before use.

v) Bovine serum albumin (250µg/ml)

b) Procedure

To 0.2ml of appropriately diluted sample was added 5ml of alkaline copper tartarate. Mixed well and the contents were allowed to stand for 10 minutes at room temperature. Then

0.5ml of Folin's reagent was added rapidly, mixed and contents were allowed to stand for 30 minutes. Intensity of blue color so developed was read at 620 nm. Amount of protein in sample was calculated from standard curve prepared simultaneously with bovine serum albumin (25-250µg/ml) as standard.

3.6 Optimization of protein extraction using Response surface methodology

3.6.1 Experimental design

For carrying out the study on optimization of extraction conditions of protein, an experimental plan was chosen from the family of three variables as suggested by Box and Behnken (1960). Using Box- Behnken design for three factors, 17 runs were planned for experimentation. The design was taken from response surface designs and it fulfills most of the requirements needed for optimization of the protein extraction conditions.

Design Summary

File Version	**9.0.6.2**		
Study Type	Response Surface	**Runs**	17
Design Type	Box-Behnken	**Blocks No**	No blocks
Design Model	Quadratic	**Build Time (ms)**	1.80

Following range of variables as bran/water ratio, time and pH were selected for experimental design: 0.1-0.25, 30-60 min extraction and pH 7-12 and two responses i.e. yield and % protein recorded.

Independent Variables	Symbol		Levels	
	Coded	Un-coded	Coded	Un-coded
B/W ratio	X_1	A	1	0.25
			0	0.175
			-1	0.1
Time(min)	X_2	B	1	60
			0	45
			-1	30
pH	X_3	C	1	12
			0	9.5
			-1	7

3.6.2 Optimization of process parameters

Response surface methodology was applied to the experimental data using a commercial statistical package, Design-Expert version 9.0.6 (Statease Inc, Minneapolis, USA, Trial version). The same software was used for the generation of response surface plots,

superimposition of 3-D plots and optimization of process variables. 3-D plots were generated for different interaction for any two independent variables, while holding the value of other variable as constant (at the central value). Such three-dimensional surfaces could give accurate geometrical representation and provide useful information about the behavior of the system within the experimental design (Cox and Cochran 1964, Montgomery 2004). The optimization of the protein extraction process aimed at finding the levels of independent variables viz. bran/water ratio, extraction time and pH of solution which could give maximum possible yield and protein yield.

3.7 Isolation and characterization of protein fractions

3.7.1 Isolation of protein fraction

Sequential extraction of water soluble (WS) and salt soluble (SS) fractions from DRBP were carried out using 2% NaCl, followed by extraction with 70% ethanol to obtain ethanol soluble (ES) fraction. The alkaline soluble (AS) fraction was extracted from the residue by using 0.05 M NaOH. All the extracts were extensively dialysed against water at 4^0 C. The WS and SS fractions were separated from the 2% NaCl extract by centrifugation after dialysis, with the supernatant and sediment collected respectively (as shown in figure 1). All protein extracts were centrifuged at 5600g for 30 min and then freeze dryed separately. The protein content was then determined using the method of Lowry *et al* (1951).

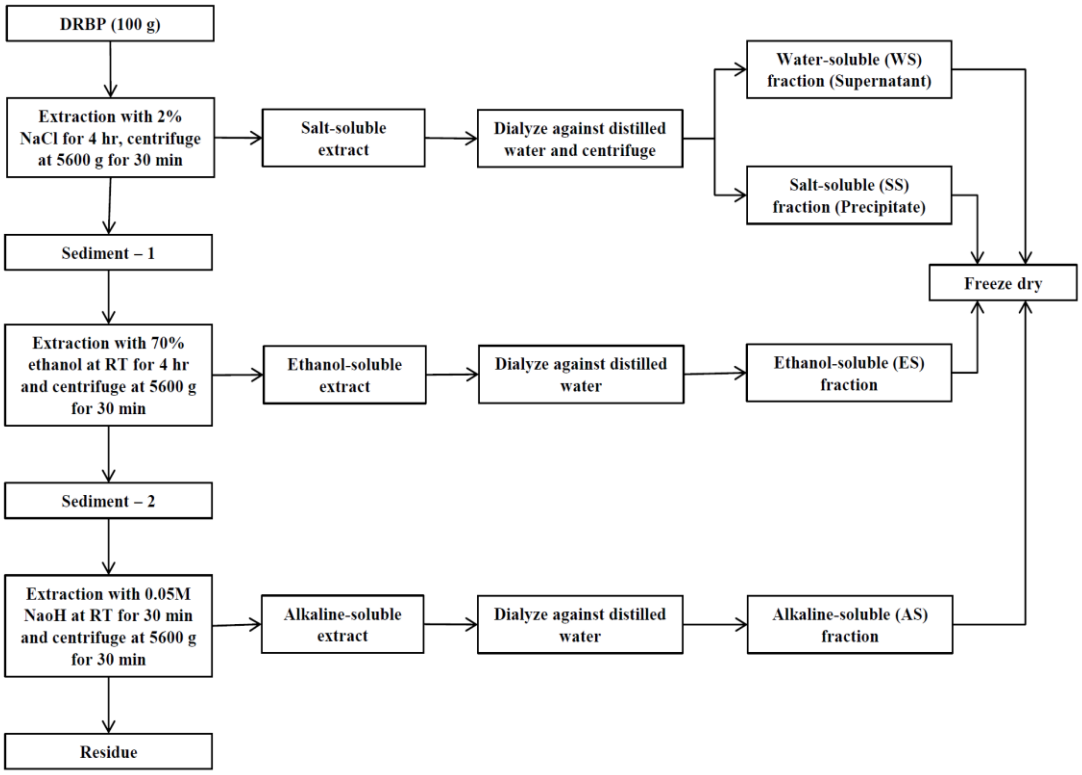

Figure 1: Scheme of sequential extraction of deoiled rice bran protein fractions from deoiled rice bran protein isolates (Adebiyi and Aluko 2011)

3.7.2 Determination of antioxidative activity of protein fractions (Satio *et al* 2003)

Radical scavenging activity was determined by measuring the spectrophotometre changes of the ABTS radical cation, using Trolox as standard. Antioxidant activity was expressed as Trolox equivalent per mg dry weight.

3.7.3 SDS-PAGE of protein fractions

SDS-polyacrylamide gel electrophoresis

3.7.3.1 Reagents

1. Stock Solution

a) Acrylamide stock (30%)

Acrylamide	29.2 g
Bisacrylamide	0.8 g
Water	100 ml

Volume was made upto 100 ml with distilled water

b) Separating gel buffer

1.5 M Tris-HCI – 18.17 g of Tris base was dissolved in 80 ml of distilled water. The pH was adjusted with 1 N HCI to 8.8 and volume was made to 100 ml.

c) Stacking gel buffer

0.5 M Tris HCI (pH 6.8) – 6 g of Tris base was dissolved in 80 ml of distilled water. The pH was adjusted with 1 N HCI to 6.8 and volume was made to 100 ml.

d) 5X running buffer

i. Tris base 7.55 g

ii. Glycine 36 g

Dissolved in 450 ml of distilled water and the pH was adjusted to 8.2 with 1 N HCI and total volume was made to 500 ml with distilled water. 1X buffer was used for running the electrophoresis. 1 g SDS per litre was added to 1X buffer before use.

e) 10% SDS

f) N, N, N, N - Tetramethyl ethylene diamine (TEMED)

g) 10% Ammonium persulphate (APS) – Freshly prepared

2. Working Solutions

a) Resolving gel (12%) was prepared by mixing the following solutions

1. 8.0 ml of 30% acrylamide
2. 5.0 ml of Tris HCI (pH 8.8)

3. 0.2 ml of 10% SDS
4. 6.6 ml of glass distilled water
5. 0.01 ml of TEMED

b) Stacking gel (5%) was prepared by mixing the following solutions

1. 1.02 ml of 30% acrylamide
2. 1.5 ml of Tris HCI (pH 6.8)
3. 0.06 ml of 10% SDS
4. 3.33 ml of glass distilled water
5. 0.01 ml of TEMED

The contents were stirred. 0.2 ml and 0.06 ml of freshly prepared 10% ammonium persulphate (APS) was added to the resolving and stacking gels, respectively.

c) Sample buffer was prepared by mixing the following solutions

1. 1.2 ml of 0.5 M Tris HCI (pH 6.8)
2. 2.0 ml of 10% SDS
3. 1.0 ml of glycerol
4. 4.8 ml of glass distilled water
5. 0.5 ml of 0.5% bromophenol blue
6. 0.5 ml of β-mercaptoethanol

β-mercaptoethanol was added just before use.

3.7.3.2 Cleaning of the electrophoresis apparatus and preparation of gel

The surfaces of the plates were washed firstly with detergent and dried. The dried glass plates were clumped together using spacers and fixed in gel casting apparatus. The assembled plates were checked for leakage with distilled water.

The resolving gel solution was immediately poured into the gel mould upto the level of 1 cm below the position of sample wells. The surface of polymerizing gel was overlaid with 0.5 ml of water using micropipette. The separating gel was left to polymerize for 15-30 minutes. The water was poured off from the top of separating gel. After which the mould was filled with the stacking gel solution up to the top of the plates and the comb of required thickness was inserted. The stacking gel was left to polymerize. After about one hour, the comb was carefully removed without disturbing the wells. The wells were washed twice with the reservoir buffer. Care was taken to remove bits of polymerized gel present in the wells.

3.7.3.3 Sample preparation, loading of samples and running of electrophoresis

Protein samples were mixed with equal volume of sample buffer. Placed the gel containing plates in vertical slab gel electrophoresis apparatus containing running buffer in

such a way so that no air bubbles are formed at the bottom of the gel. The upper reservoir was filled with running buffer. Sample containing 100 µg of protein was loaded in each well with the help of micropipette or syringe. After loading the samples, the electrodes were connected to DC powerpack and current was adjusted to 1.5 mA per cm. Electrophoresis was continued until the dye (bromophenol blue) reached 1 cm from the bottom of the gel. The standard protein ladder with Molecular range 29-200 kD was run alongwith the sample.

3.7.3.4 Staining and destaining of proteins

After the termination of electrophoresis, the gel plates were removed and carefully separated from each other. Made a cut on the gel corner for marking the position of samples. The gel was then placed in staining solution for 4-5 hours and destained with several changes of destaining solution. The gels were preserved in 7 percent acetic acid solution.

3.8 Preparation of cookies, muffins and their analysis

3.8.1 Preparation of Cookies and muffins

3.8.1.1 Preparation of Cookies using DRB and DRBP

The DRB was incorporated on cookies at 10%, 15% and 20% level by replacing the wheat flour and were compared with control sample.

Table 1: Composition of cookies

Ingredients	Control	10%	15%	20%
DRB	0	10	15	20
Wheat flour	100	90	85	80
Sugar	55	55	55	55
Fat	45	45	45	45
Salt	0.5	0.5	0.5	0.5
Sodium bicarbonate	1	1	1	1

The dough was sheeted, cut into circular shape and baked for 10 min at 400^{o} F.

For incorporation of DRBP wheat flour was replaced with wheat flour at 1%, 2% and 3% level of replacement

3.8.1.2 Preparation of Muffins

The DRB was incorporated on muffins at 5%, 10%, 15% level and were compared with control sample.

Table 2: Composition of muffins

Ingredients(g)	Control	5%	10%	15%
DRB	0	5	10	15
Flour	100	95	90	85
Sugar	90	90	90	90
Egg	220	220	220	220
Fat	60	60	60	60
Sodium bicarbonate	2.5	2.5	2.5	2.5
Skim milk	1	1	1	1
Baking soda	2.5	2.5	2.5	2.5
Total	476	476	476	476

3.8.2 Proximate analysis of cookies and muffins

Proximate analysis of cookies and muffins were done as per procedure described in section 3.3.

3.8.3 Sensory analysis

The developed cookies and muffins were sensory evaluated for appearance, color, flavor, texture and overall acceptability by a panel of judges on a nine point hedonic scale with following individual scores: liked extremely-9, liked very,much-8 liked moderately-7, liked slightly-6, neither liked nor disliked-5, disliked slightly-4, disliked moderately-3, disliked very much-2, and disliked extremely-1 to find out the most suitable composition of cookies for commercialization.

3.8.4 Spread ratio of cookies

To measure the diameter of cookies, four samples were placed next to one another and total diameter was measured. All of them were then rotated at 90^0 and the new diameter was measured. The average of the two measurements divided by four was taken as the final diameter of the cookie. Thickness as measured by stacking the cookies one above the others and restacking four times.

The spread ratio was calculated using the formula: $\frac{\text{diameter of cookies}}{\text{height of cookies}}$

3.8.5 Specific volume of muffins

Specific volume of muffins was performed based on substitution of sesame seeds by muffin in a certain volume container.

$$\text{Specific volume} = \frac{\text{Vol. of sesame seeds in a container} - \text{Vol. of sesame seeds in a container with muffins}}{\text{Weight of muffins}}$$

CHAPTER IV

RESULTS AND DISCUSSION

The results and discussion are presented under following sub-headings:

4.1 Proximate analysis of deoiled rice bran

4.2 Extraction of protein concentrate by chemical method

4.3 Extraction of protein concentrate by enzymatic method

4.4 Fractionation and characterization of protein concentrate

4.5 Utilization of deoiled rice bran in wheat based food products

4.1 Proximate analysis of deoiled rice bran

The data given in table 3 and figure 2 depict the chemical composition of deoiled rice bran. It contained 12.1 % moisture, 12.52 %, crude fiber and 14.92 % crude protein respectively. Total protein content (14.92%) was in the range of 10-38% protein obtained from the other work in rice bran using various methods (Juliano 1985, Shih *et al* 1999, Parrado *et al* 2006 and Ali *et al* 2010). Deoiled rice bran contained 9.7% ash and 0.6% silica. Jiamyangyuen *et al* (2005) reported that crude fiber in deoiled rice bran was 6.03%, crude protein 13.89% and ash content was 10.13%. The difference in proximate composition might be due to the varietal difference among deoiled rice bran.

Table 3: Proximate chemical composition of DRB

Parameter	Raw DRB
Moisture (%)	12.1±0.10
Crude Protein (%)	14.92±1.02
Crude Fibre (%)	12.52±0.52
Ash (%)	9.70±0.30
Silica (%)	0.6±1.02

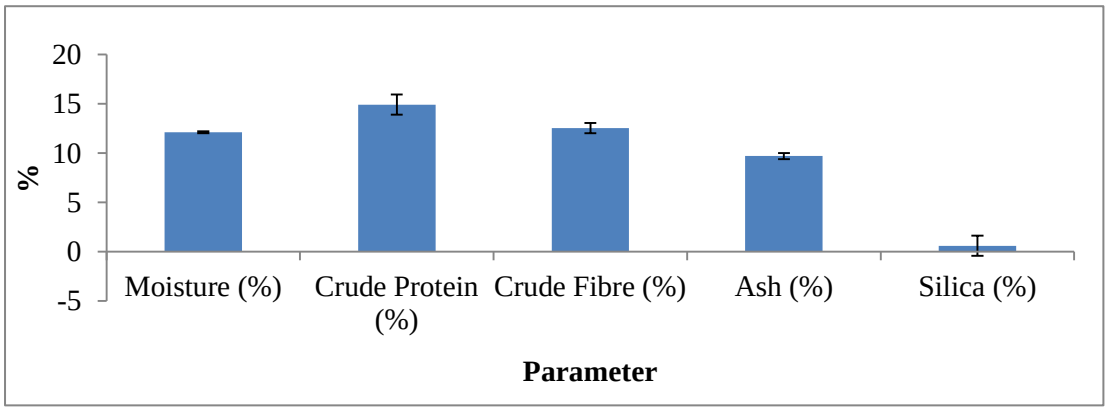

Figure 2: Proximate chemical composition of DRB

4.2 Extraction of protein concentrate by chemical method

4.2.1 Experimental design

An experimental design was drawn using software Dx9 9.0.6 trial version. Design was based on three independent variables viz. time (30-60 min), B/W (0.1-0.25) and pH (7-12.0) and two responses viz. yield % and protein %.

4.2.2 Experimental design for protein

Table 4 showed yield % and protein % in extracted protein concentrate extracted from deoiled rice bran. Under different experimental combinations, a wide variation in all the responses was observed i.e. 5.3 to 9.56 % for yield of protein concentrate with 51.25 -83.75% protein content. Maximum yield of protein concentrate from deoiled rice bran was obtained when extracted with B/W of 0.25 for 45 min at pH 12.0, whereas maximum protein (%) was found with B/W of 0.175, time 45 min and pH 9.5 indicating that yield and properties of protein concentrates were affected by extraction conditions. The protein content increased linearly from pH 7 to 9.5 and then decreased at 12.0. According to Jiamyangyuen *et al* (2005), at high pH some non- protein nitrogen could solublize and contribute to protein quality and quantity.

Table 4: Effect of different factors on yield and protein conc. of protein extracted from deoiled rice bran using chemical method

Run	Independent variables			Responses	
	A:pH	B:Time	C:B/R	Yield (%)	Protein (%)
1	7	30	0.175	7.05	62.46
2	7	45	0.25	7.31	62.77
3	7	45	0.1	7.46	62.85
4	7	60	0.175	7.58	63.04
5	9.5	30	0.25	8.13	65.00
6	9.5	30	0.1	8.39	78.75
7	9.5	45	0.175	8.47	83.39
8	9.5	45	0.175	8.63	83.75
9	9.5	45	0.175	8.68	82.37
10	9.5	45	0.175	8.58	82.65.
11	9.5	45	0.175	8.61	79.96.
12	9.5	60	0.25	6.31	72.50
13	9.5	60	0.1	7.68	61.25
14	12	30	0.175	9.47	77.50
15	12	45	0.25	9.56	68.75
16	12	45	0.1	11.7	58.75
17	12	60	0.175	5.3	51.25

Regression analyses were carried out to fit the mathematical models to the experimental data. The quadratic model obtained from regression analysis for yield % and protein % in terms of un-coded levels of the variables was developed as follows:

Yield (%) = +8.60 +0.95 *A -0.78 * B -0.63 * C -0.92 * AB -0.50 * AC -0.51 * BC +0.31 * A^2 -1.30 * B^2 +0.10 * C^2

Protein Conc. = +83.75 +3.30 * A -4.46 * B -1.73 * C -1.40 * AB +2.52 * AC +0.94 * BC -7.83 * A^2 -7.05 * B^2 -12.64 * C^2

Table 5 showed the sum of square values and p-values for different responses viz. yield of protein concentrate and protein content in concentrate . Values of 'p' less than 0.0500 indicate model terms are significant at 5% level of significance.

Table 5: ANOVA for yield and protein % of protein concentrate extracted from deoiled rice bran with chemical method

Parameter	Sum of Squares	
	Yield (%)	**Protein (%)**
Model	28.16 (0.00146)*	1567.75 (0.0375)*
pH(A)	7.30 (0.0077)*	86.99 (0.1944)
Time(B)	4.88 (0.0192)*	159.04 (0.0935)
B/W(C)	3.14 (0.0457)*	23.91 (0.4764)
AB	3.40 (0.0394)*	7.78 (0.6806)
AC	0.99 (0.2151)	25.40 (0.4634)
BC	1.04 (*0.2050*)	3.52 (0.7813)
A^2	0.40 (0.4146)	258.06 (0.0427)*
B^2	7.16 (0.0080)*	209.05 (0.0614)
C^2	0.043 (0.7847)	672.85 (0.0052)*
R^2	0.8830	0.8413
Std. Dev.	0.73	6.50

*indicate significant effect
Data in parenthesis represent the p-value

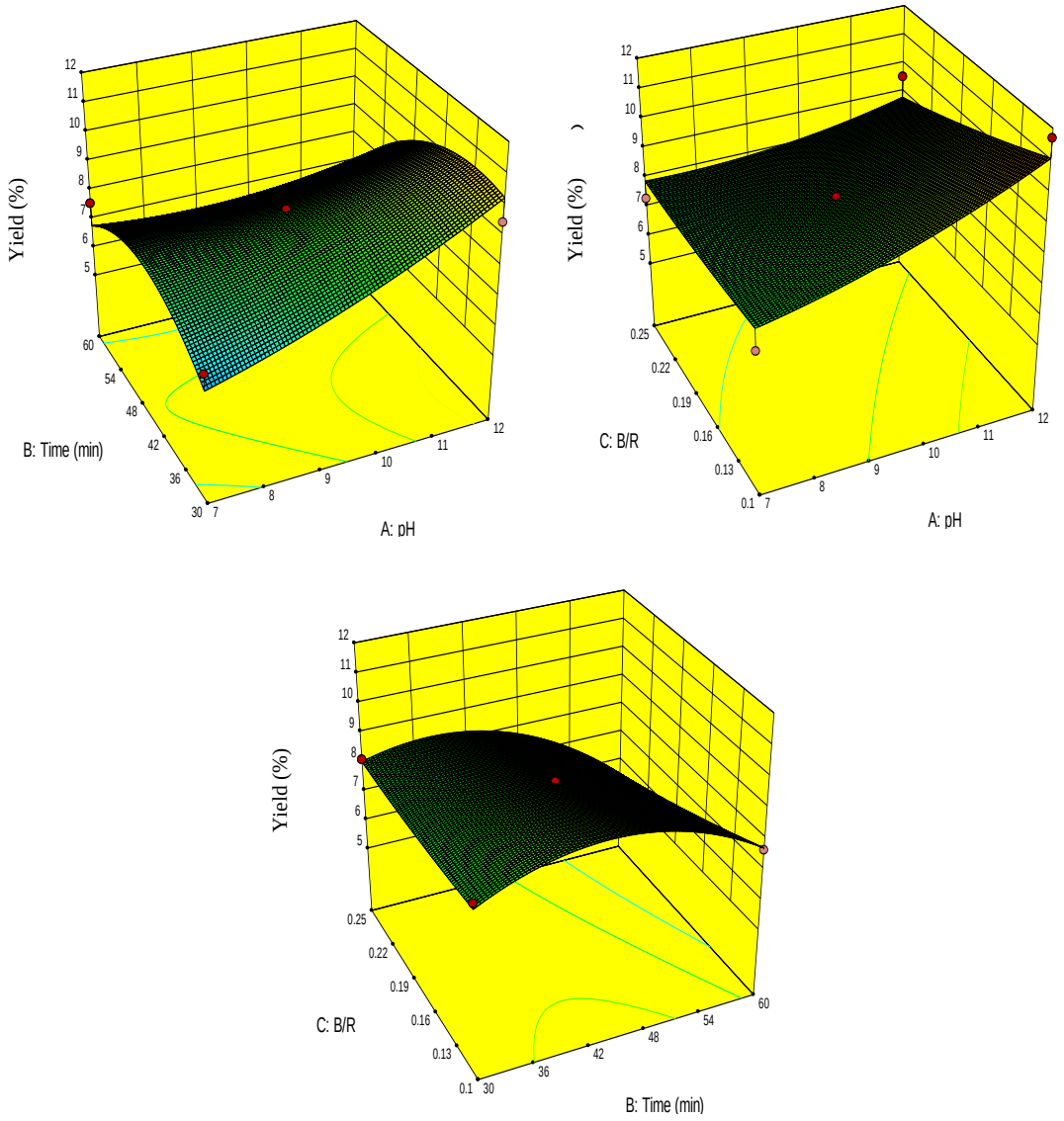

Figure 3: Effect of variables on yield of protein concentrate from DRB

Effect of variables on yield of protein

Figure 3 showed that the yield of extracted protein concentrate extracted increased with the increase in time and then decreased. Yield of protein concentrate increased with increase in pH and then declined with further increase. Lysinoalanine, a possible toxic product (Cheftel *et al*, 1985), is identified during strong alkali extraction between pH 10-12 (Degroot and Slump, 1969). Variables i.e. pH, temperature and bran/water ratio had an significant effect on yield of protein. Among all the variables least p-value found for pH. So, pH of extraction solvent witnessed most significant effect on protein concentrate yield from deoiled rice bran followed by time of extraction and B/W ratio as shown in table 5. It was also significantly affected by time: pH interaction ($p<0.05$).

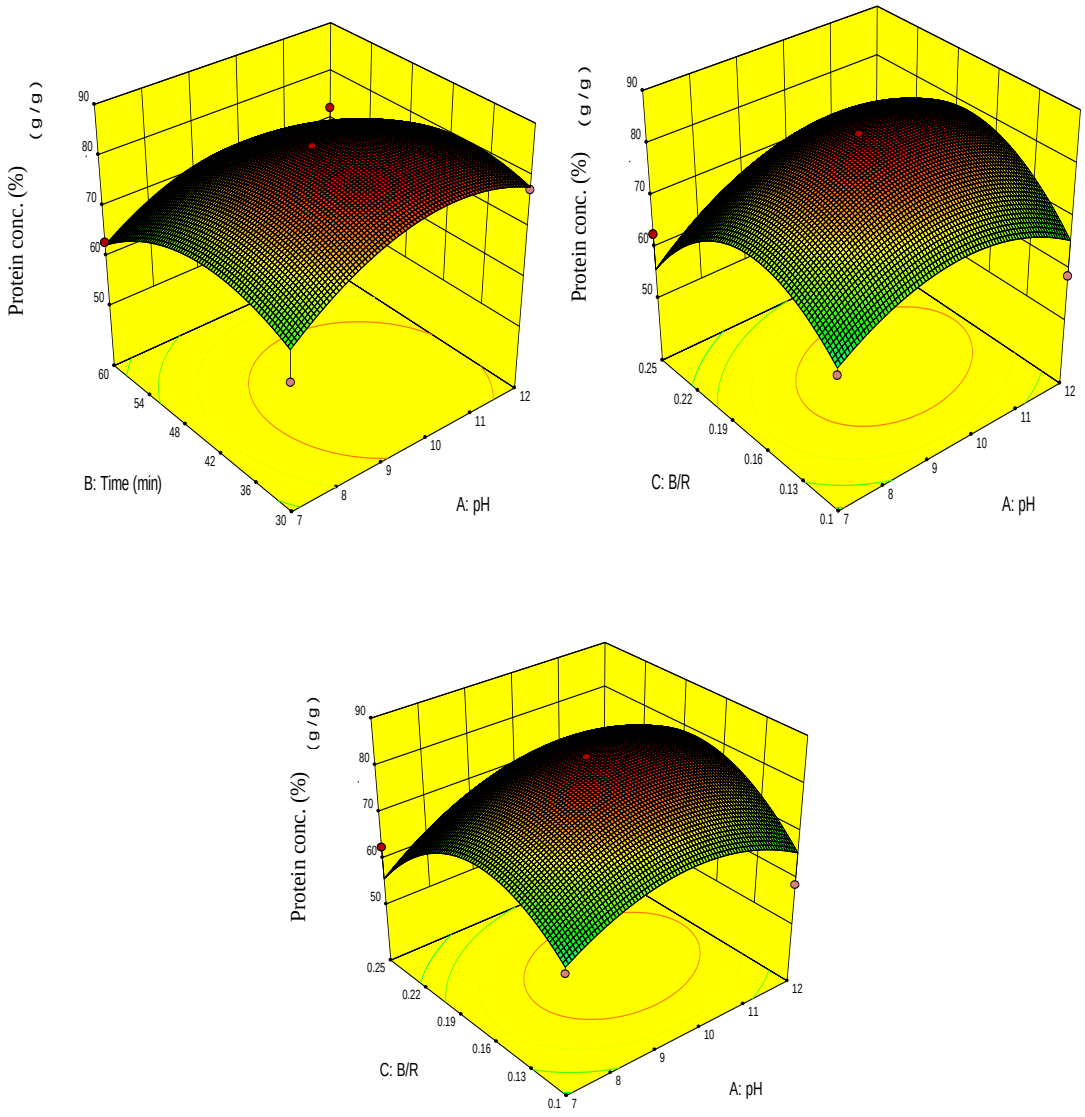

Figure 4: Effect of variables on protein % of extracted protein concentrate

Effect of variables on protein content of extracted protein concentrate

Protein content of extracted protein concentrate first increased with increase in pH and increase in time of extraction and then declined with further increase in these variables (Figure 4). It was effected significantly by pH:pH interaction ($p<0.05$). pH, time and B/W ratio had no significant effect on on protein content of extracted protein concentrate (Table 5).

4.2.3 Optimization of results

Optimization can be defined as the processing conditions that give the optimum (maximum or minimum) value of a function of certain decided variables subject to constraints that are imposed. Optimization may be the process maximizing a desired quantity or minimizing an undesired one. The values of the processing variables that produce the desired optimum value are called optimum conditions.

Table 6: Optimized vs experimental values of yield and physicochemical properties of protein concentrate extracted from deoiled rice bran with chemical method

	B/W	Time(m)	pH	Yield (%)	Protein (%)
Optimized	1/6	45	9.5	8.63	83.75
Experimental	1/6	45	9.5	8.75	84.12
Variation %	----	----	----	1.39	0.44

The optimum process parameters obtained by computer generated response surfaces, canonical analysis and 3D- plot interpretation were: 0.175 B/W, 9.5 pH of extraction solvent solution and 45 min of extraction time which gave protein concentrate yield of 8.63% and protein content 83.75 of protein concentrate. The experimental results were found to be close to optimized selected value with a variation 1.39% in yield and 0.44% protein content (Table 6). Jongjareonrak *et al* (2015) reported that optimized conditions for extraction of rice bran proteins were NaOH concentration 0.13M at an extraction temperature of 41^0C for 170 min where they found 43% of protein yield.

4.3 Extraction of protein concentrate by enzymatic method

The effect of enzyme amylase and protease on protein concentrate extraction from deoiled rice bran are shown in table 7 and 8. Addition of 0, 700, 1400, 2100 units of amylase to 100g deoiled rice bran resulted in 7.08%, 10.0%, 12.87%, 13.5% yield of protein concentrate respectively with 57.29%, 62.94%, 65.86% and 58.46% protein content respectively. Carbohydrases have been used for protein extraction from rice bran since 1997(Ansharaullah *et al* 1997, Wang *et al* 1999). Increase in the yield and recovery, disintegration of extraction cell wall tissues by enzymes as carbohydrases were thought to be involved in disintegration of cell wall tissue, facilitating protein. The treatment of α-amylase, glucomylase and then with cellulose and xylanase resulted in the 91% protein content in protein concentrate of rice flour (Shih and Diagle,2000). Adding above 1400 units of amylase had non-significant effect on protein yield while there was decrease in protein recovery (Table 7 and figure 5). Ansharullah *et al* (1997) reported that viscozyme, a cellulase, extracted more than 50% of protein from nonheat-stabilized rice bran under optimal pH, temperature, and extraction time. Wang *et al* (1999) prepared 92.0% of protein isolate with 74.6% yield (recovery) from non defatted rice bran using enzyme phytase and xylase. Tang *et al* (2003) reported that viscozyme and celluclast extracted 28.5% and 12% protein respectively which were lower than the value when extracted using amylase.

Table 7: Effect of varying levels of enzyme amylase on extraction of protein concentrate from deoiled rice bran

Amylase Units	Yield (%)	Protein (%)
0	7.08±0.34	57.29±3.6
700	10.0±0.23	62.94±2.5
1400	12.87±0.65	65.86±2.8
2100	13.5±0.54	58.46±2.3

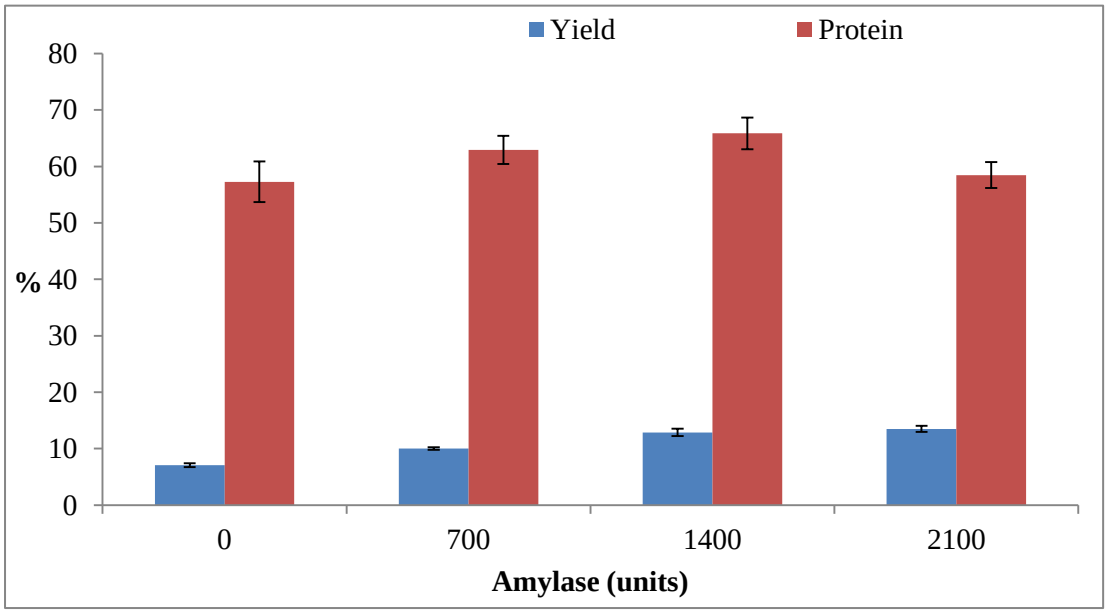

Figure 5: Effect of varying levels of enzyme amylase on extraction of protein concentrate from deoiled rice bran

Hamada (2000) reported that proteases could significantly increase protein extraction from nonheat-stabilised defatted rice bran. Addition of 0.9 unit to 2.7 units of enzyme protease resulted in gradual increase in yield of protein concentrate, however this % protein in deoiled rice bran protein concentrate increased only upto addition of 1.8 unit of protease (Table 8 and figure 6). Proteases have been used to enhance recovery of rice bran proteins from about 60% to 93% and to obtain a wide range of protein hydrolysates. Treatment of rice bran protease allowed generation of protein hydrolysates that possessed appropriate peptide chain length. At higher units, the decrease in protein % might be due to higher degree of hydrolysis. Zhang *et al* (2012) reported that yield of protein from heat stabilized defatted rice bran was 32.9% by alkaline extraction and 44.79% when alcalase 2.4% is added after alkaline extraction.

Table 8: Effect of varying levels of enzyme protease units on extraction of protein concentrate from deoiled rice bran

Protease (units)	Yield (%)	Protein (%)
0.0	7.08±0.34	65.0±3.0
0.9	11.8±0.33	71.33±4.0
1.8	12.7±0.55	72.86±3.5
2.7	15.6±0.66	64.10±2.4

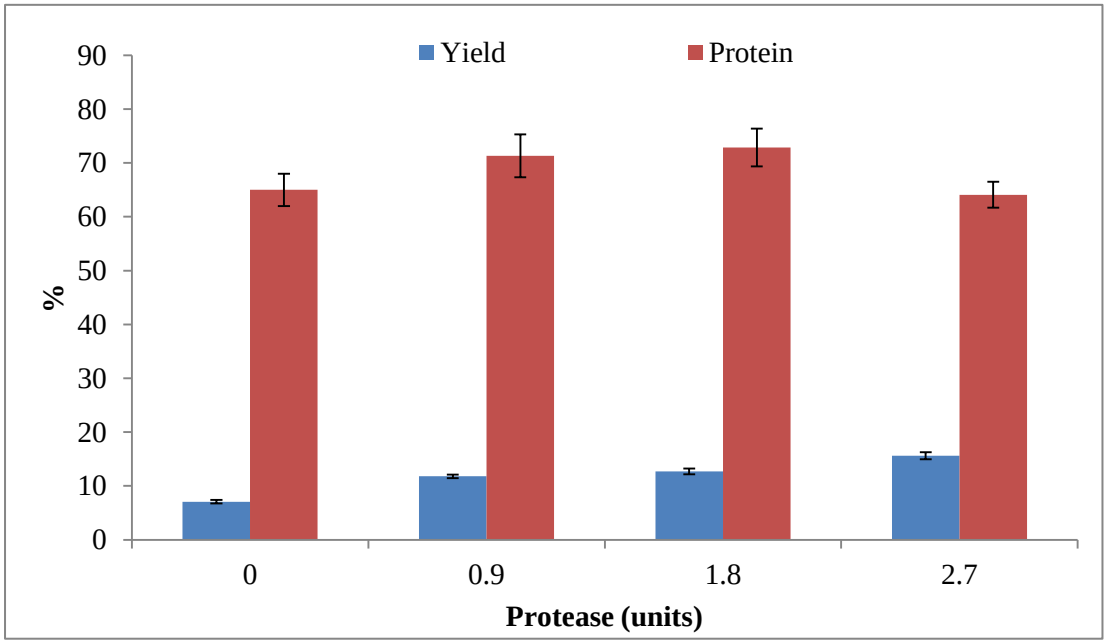

Figure 6: Effect of varying levels of enzyme protease units on extraction of protein concentrate from deoiled rice bran

4.4 Fractionation and characterization of protein concentrate

Yield of protein fractions

Table 9 and figure 7 shows the relative proportions of DRBP fractionated according to their solubility in different solvent systems, which has been illustrated in figure 1 of Materials and Method section. Yield is reported as gross weight and was obtained as the percentage ratio of the freeze-dried protein fraction to the original weight of protein concentrate (Sathe & Venkatachalam, 2007). The glutelin fraction was the predominant protein fraction (46.82%) followed by albumins fraction (37.23%), globulin fraction (20.27%) and prolamin fraction (1.18%). However, Cagampang *et al* (1966) found albumins and globulins as a major protein of the rice bran samples. There is possibility of the yield being affected by the original protein precipitate preparation method. Table (9) also shows the

protein content of the protein fractions and the water soluble (WS) fraction had the highest value (92.45%), followed by salt soluble (SS) fraction (86.83%) and alkaline soluble (AS) fraction (83.8 %) while ethanol soluble (ES) fraction had the significantly lowest ($p < 0.05$) value of (76.6%). The relatively low protein content of ES fraction might be due to its low solubility in the NaOH solution that was used in the lowry assay method and also the presence of non-protein components such as polyphenols that might have been extracted by the alcoholic solution used during fractionation (Adebiyi and Aluko, 2011).

Table 9: Yield and protein content of deoiled protein fractions isolated from deoiled rice bran protein concentrate

Protein Fraction	Yield/ 100 g DRBP	Protein % in fractions
WS (albumins)	35.23±1.52	92.45±3.86
SS (globulins)	19.17±0.96	86.83±2.65
AS (glutelin)	44.31±1.73	83.8±3.23
ES (prolamin)	1.18±0.13	76.6±1.3

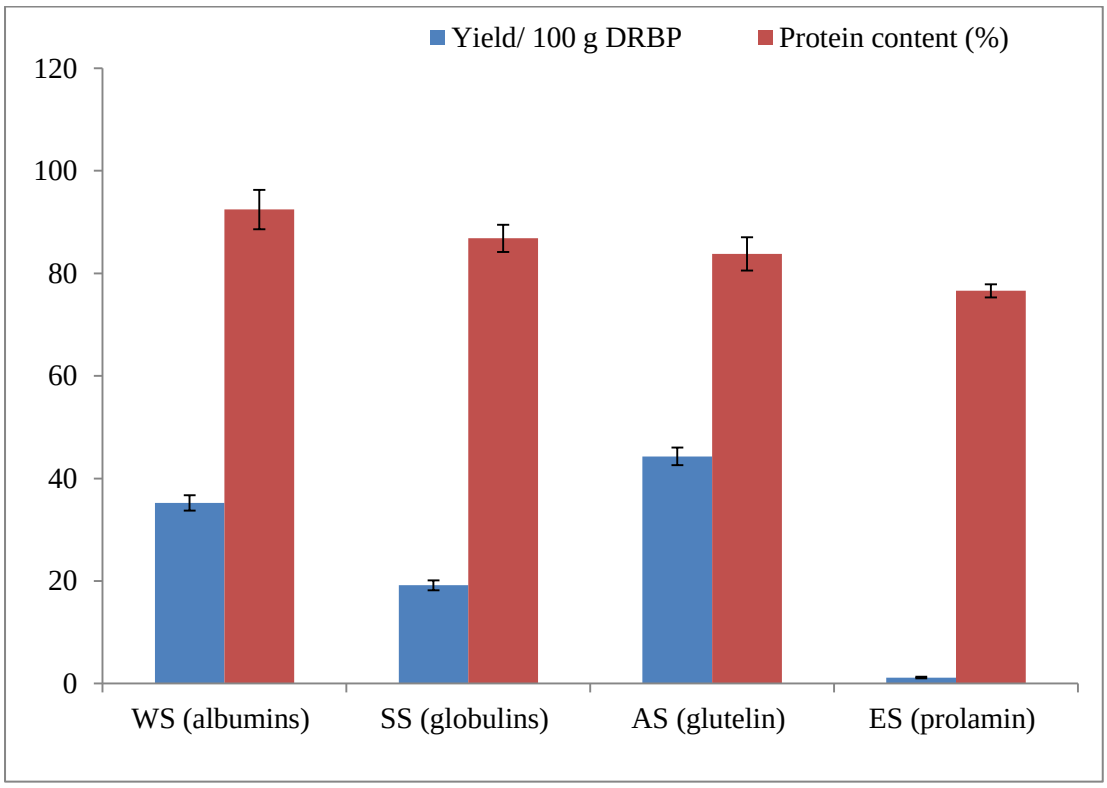

Figure 7: Yield and protein content of deoiled protein fractions isolated from deoiled rice bran protein concentrate

SDS-PAGE of protein concentrate fractions

The water soluble (WS), salt soluble (SS) and alkaline soluble (AS) fractions have slightly different polypeptide composition profiles and their bands were found between 14-66 kDa regions. The water soluble (WS) and salt soluble (SS) fraction appear as smears of bands between 43 and 66 kDa regions. The ethanol soluble (ES) fraction was not properly separated into distinguishable bands, probably as a result of limited solubility in the electrophoresis reagent (Figure 8). Hamada (2000) reported the molecular weights of rice bran albumin, globulin, glutelin and prolamin to be in the range 10-100, 10-150, 33-150 and 25-100 kDa, respectively. Low molecular weight range had been reported by earlier workers on RBP and rice flour protein: rice flour albumin 60 kDa; rice flour globulin12-20 kDa (Ju *et al* 2001), rice flour glutelin 21-28 kDa (Mawal *et al* 1990), rice bran glutelin 16 kDa (Landers and Hamaker 1994) and rice flour prolamin 10-16 kDa (Losso *et al* 2003). These discrepancies might be due to the heterogeneous nature of polypeptides in rice flour and rice bran. Different varieties of rice, the methods employed for isolation of RBPF and characterization might also be responsible (Adebiyi *et al* 2009).

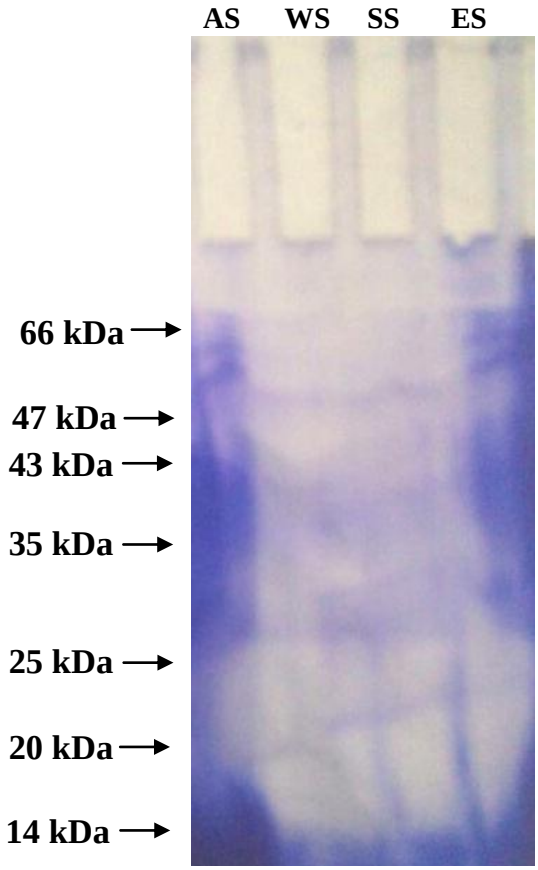

Figure 8: SDS-PAGE of protein concentrate fractions

Antioxidant activity of protein concentrate fractions

Antioxidant activity of deoiled rice bran and protein fractions of deoiled rice bran protein concentrates had been shown in table 10 and figure 8. The albumin fraction had the highest antioxidant activity (48.81 mgTE/gDw) followed by glutelin fraction (27.35 mgTE/gDw), globulin fraction had antioxidant activity of 10.18 mgTE/gDw whereas lowest antioxidant activity was found in prolamin fraction i.e. 4.36 mgTE/gDw. High antioxidant activity of albumin fraction might be caused by high phytic acid content. Phytic acid is reported to be natural antioxidant (Graf *et al* 2006) and was found in albumin fraction of deoiled rice bran protein (Adebiyi *et al* 2009).

Table 10: Anti-oxidative activity of deoiled DRBP protein fractions

Protein Fraction	TEAC (mgTE/gDw)
Total	32.41±0.41
WS (Albumins)	48.81±1.32
SS (Globulins)	10.18±0.51
AS (Glutelin)	27.35±0.83
ES (Prolamin)	4.36±0.36

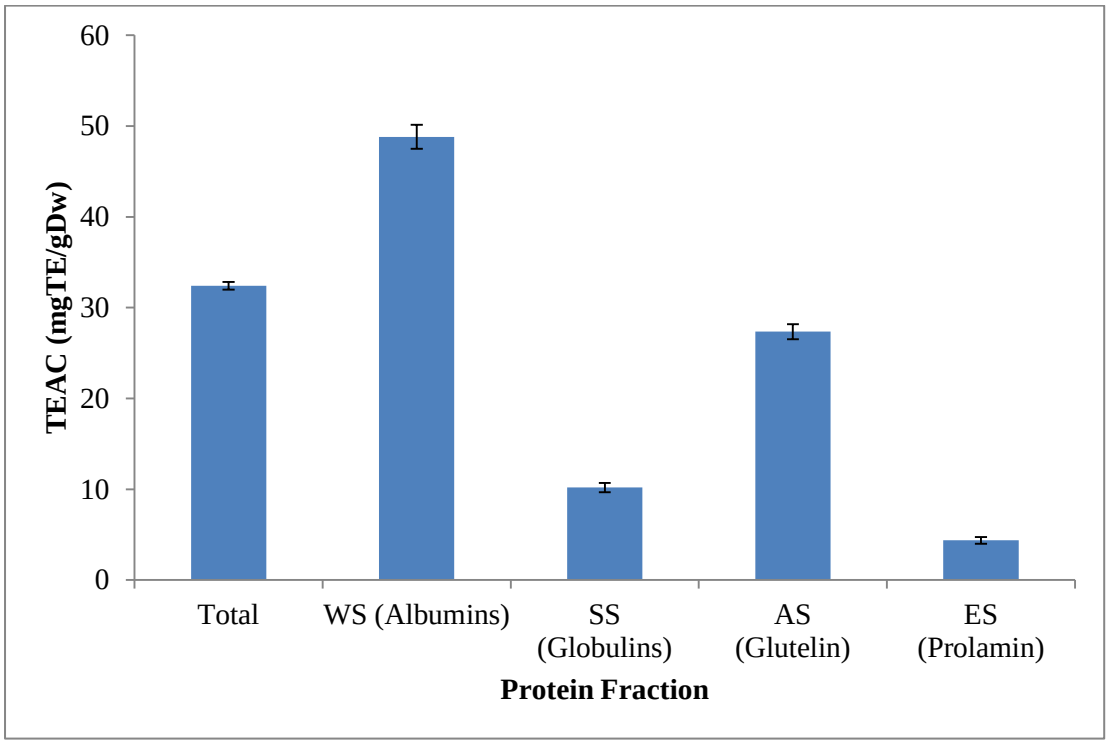

Figure 9: Anti-oxidative activity of deoiled DRBP protein fractions

4.5 Utilization of deoiled rice bran in wheat based food products

Proximate composition of cookies prepared by using deoiled rice bran

The deoiled rice bran was incorporated in cookies at 10, 15 and 20% substitution level by replacing wheat flour and was compared with control for chemical composition as well as for sensory evaluation.

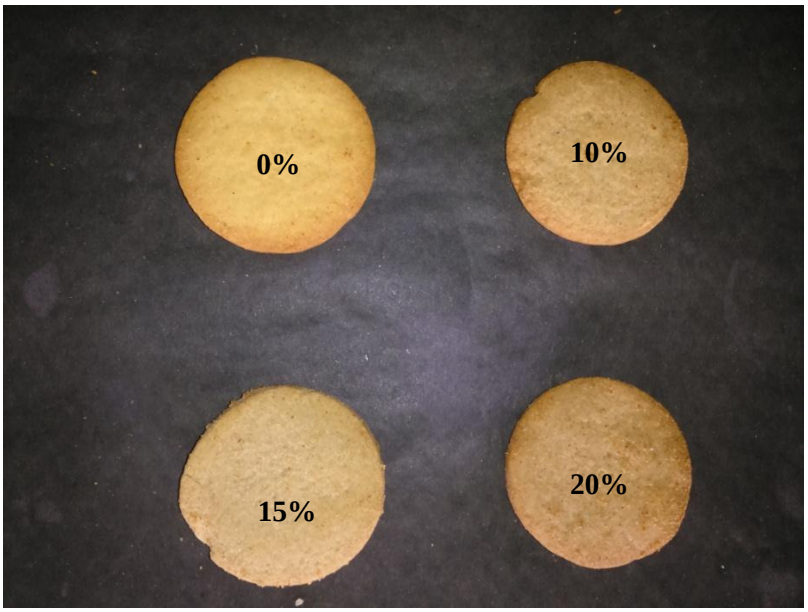

Figure 10: Cookies incorporated with deoiled rice bran

The proximate composition of cookies at 10%, 15% and 20% level of substitution of deoiled rice bran shown in table 11 and figure 11 which showed an increase in protein from (8% to 9.70%) and ash content (0.60% to 2.26) and in fibre (2.3% to 4.74%), in deoiled rice bran substituted cookies. However difference in fat content of all the samples was not statistically significant. The protein, fibre and ash content increased with the increase in the amount of RBP substitution which might be due to the fact that rice bran is a good source of fibre (Hamid and Luen, 2000) as well as being a considerable source of protein. 10%-20% replacement of wheat flour with DRB had also recommended by Sharif *et al* (2009)

Table 11: Proximate composition of cookies prepared by using deoiled rice bran

Incorporation of DRB (%)	Crude protein (%)	Fat (%)	Crude Fibre (%)	Ash (%)
Control (0%)	8.00±0.029	31.00±0.577	2.30±0.359	0.60±0.035
(10%)	8.81±0.110	31.01±0.548	3.55±0.115	1.48±0.104
(15%)	9.13±0.136	31.00±0.417	4.18±0.287	1.97±0.073
(20%)	9.70±0.087	31.05±0.475	4.74±0.311	2.26±0.117

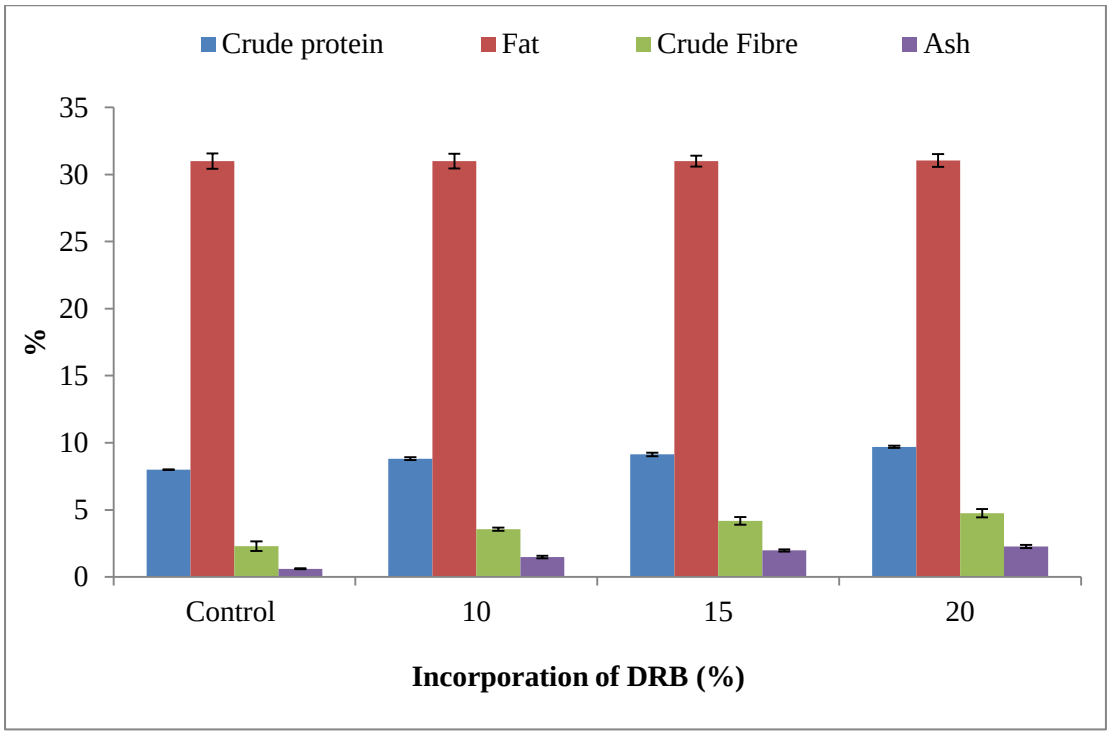

Figure 11: Proximate composition of cookies prepared by using deoiled rice bran

Table 12: Sensory Analysis of cookies incorporated with DRB

Incorporation of DRB (%)	Panelist					
	1	2	3	4	5	Overall Acceptability
Control (0%)	5.0	8.5	7.0	8.0	6.5	6.9
10%	8.5	8.0	8.0	8.0	7.8	8.1
15%	8.3	7.8	6.0	7.0	7.5	7.3
20%	7.8	7.4	7.0	6.5	7.0	7.1

Sensory evaluation revealed that cookies with 10% are palatable with adequate physical and sensory characteristics. Overall acceptability was maximum for cookies at 10% level of substitution of deoiled rice bran as shown in table 12. By progressive increase in supplementation of DRB, color of cookies turned toward darker, leading to low acceptance. At high substitution, the darker color and unfamiliar taste to panelists of 15% and 20% substituted samples could be reason for lower hedonic scores at higher substitution level.

The spread of the cookies should be according to the specifications set by the manufacturers. Too much elasticity in the gluten and dough will spring back to give thicker cookies due to smaller diameter. Supplementation of various levels of deoiled rice bran has

a significant effect on spread ratio. Increase in spread ratio contributes to better quality of cookies. The control sample had a spread ratio of 6.25 which increased to 7.8 for 10% and 7.7 for 15% and 7.3 for 20 %level of supplementation On the contrary Sekhon *et al* (1997), reported that the spread of cookies decreases with the addition of rice bran.

Table 13: Spread ratio of cookies incorporated with DRB

Incorporation of DRB (%)	Spread ratio of cookies			
Control (0%)	6.0	6.3	6.4	6.25
10%	8.2	7.8	7.4	7.8
15%	7.3	7.7	8.2	7.7
20%	6.9	8.1	7.6	7.3

Proximate composition of cookies prepared by using deoiled rice bran protein concentrate

The proximate composition of cookies at 1.0%, 2.0% and 3.0 % level of substitution of deoiled rice bran protein concentrate is shown in table 14 and figure 12 which showed an increase in protein from (8% to 10.14%), and ash content (0.60% to 0.70) in deoiled rice bran protein concentrate substituted cookies, whereas non-significant difference in fat content and non-significant decrease in fibre content was found in these cookies with respect to control.

Table 14: Proximate composition of cookies prepared by using deoiled rice bran protein concentrate

Incorporation of DRBP (%)	Crude Protein	Fat	Crude fibre	Ash
Control (0%)	8.00±0.029	31.00±0.578	2.30±0.359	0.60±0.035
1%	8.73±0.017	31.05±0.60	2.29±0.018	0.64±0.012
2%	9.37±0.04	31.02±0.55	2.27±0.043	0.67±0.009
3%	10.14±0.081	31.06±0.29	2.24±0.035	0.70±0.012

Values are the mean of 3 replications± S.E

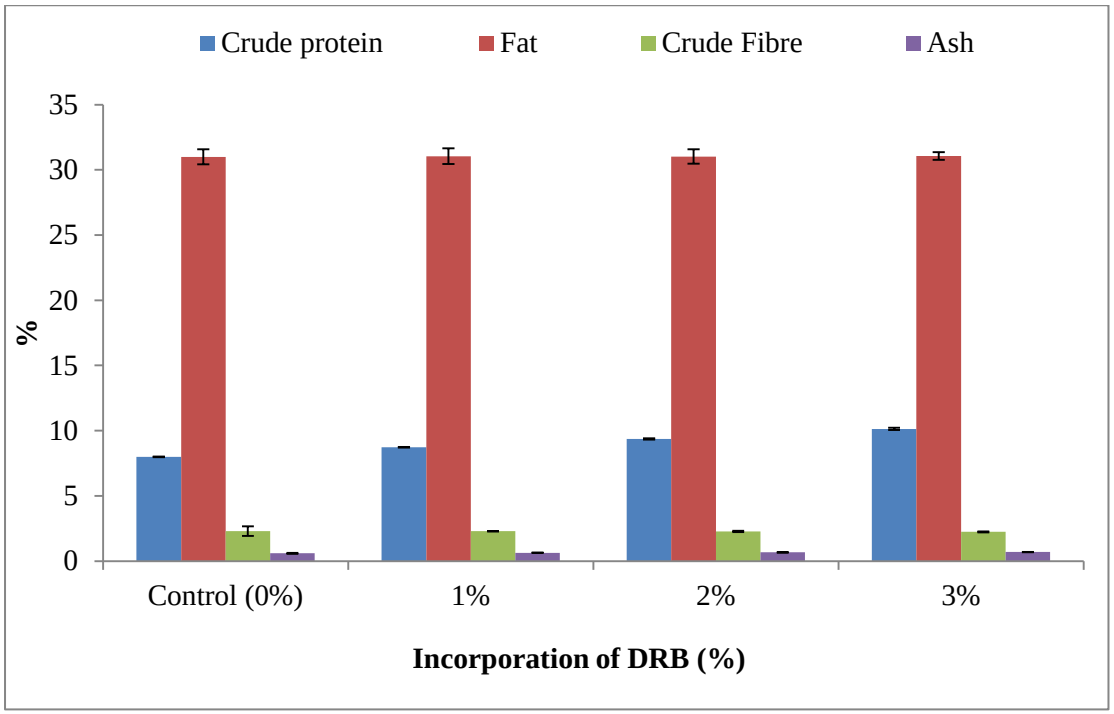

Figure 12: Proximate composition of cookies prepared by using deoiled rice bran protein concentrate

Sensory evaluation of cookies prepared by using deoiled rice bran protein concentrate

Refined flour in the cookies was substituted with deoiled rice bran protein concentrate at 1.0, 2.0 and 3.0% level (Figure 13) and sensory evaluation of cookies was done by group of semi trained panelist on a 9 point hedonic scale. The sensory evaluation revealed that cookies with deoiled rice protein concentrate (Table 15) at 1% deoiled rice bran protein concentrate were more palatable with adequate sensory characteristics. Overall acceptability was highest for cookies developed at 1% level of substitution of flour with deoiled rice bran concentrate. However, overall acceptability for cookies at 2 and 3% level of substitution of flour with deoiled rice bran concentrate was less as compared to control (Table 1) which might be due to unfamiliar taste of deoiled rice bran protein concentrate. So, even through high use of DRBPC in cookies is desirable in terms of chemical composition, sensory evaluation of product has restricted its use up to 1% level in order to maintain consumer acceptability of product. Jiamyangyuen *et al* (2005) also found that 1% level of deoiled RBPC in bread was most acceptable

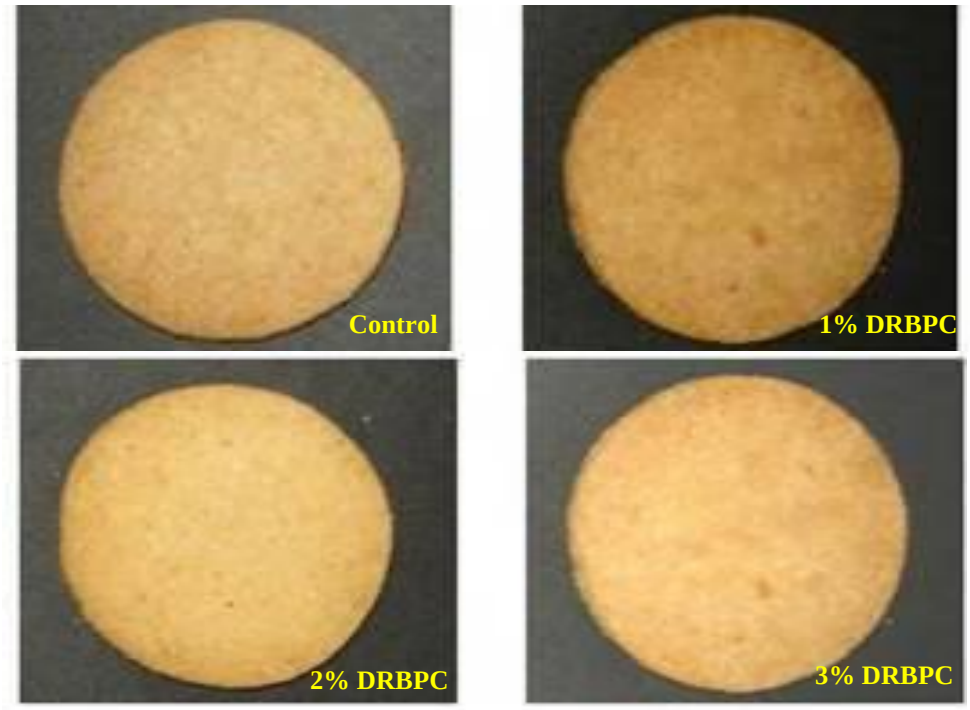

Figure 13: Cookies with deoiled rice bran protein concentrate compared with the control

Table 15: Sensory evaluation of cookies

Incorporation of DRBP (%)	Panelist					
	1	2	3	4	5	Acceptability
Control (0%)	7.0	7.5	7.5	8.0	6.5	7.3
1%	8.0	8.5	7.0	7.5	6.5	7.5
2%	7.5	7.0	7.0	7.5	6.2	7.04
3%	6.0	6.8	6.0	6.7	5.5	6.2

Sensory evaluation of muffins prepared by using deoiled rice bran

The deoiled rice bran was incorporated in muffins at 5, 10 and 15% substitution level by replacing wheat flour (Figure 14) and was compared with control for sensory evaluation. The sensory evaluation revealed that muffins with deoiled rice bran at 15% deoiled rice bran were more palatable with adequate sensory characteristics (Table 16). As compared to control muffins with deoiled rice bran had high acceptability.

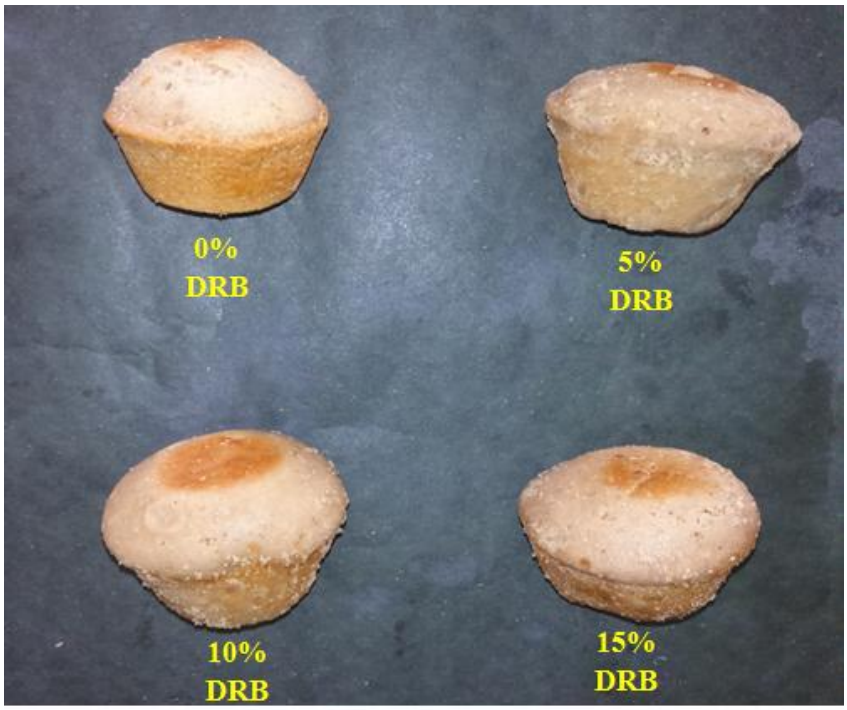

Figure 14: Muffins with deoiled rice bran

Table 16: Sensory analysis of muffins incorporated with DRB

Incorporation of DRB (%)	Panelist					
	1	2	3	4	5	Overall Acceptability
Control(0%)	5.0	8.5	7.0	8.0	6.5	6.8
5%	7.0	7.1	6.8	7.6	7.4	7.1
10%	8.3	7.8	6.0	7.0	7.5	7.6
15%	8.5	8.0	8.0	8.1	7.9	8.2

Table 17: Effect of incorporation of DRB on specific volume of muffins

Incorporation of DRB (%)	Weight (g)	Volume (ml)	Specific volume (cm^3/g)
Control (0%)	34.176	110	3.21
5%	34.408	128	3.72
10%	36.151	147	4.06
15%	37.358	174	4.65

Overall acceptability was highest for muffins developed at 15% level of substitution of flour with deoiled rice bran followed by 10% and then 5% level of substitution. Specific volume of muffins also increased with the increase in level of substitution (Table 17).

So deoiled rice bran can be successfully incorporated in cookies at 10% level and in muffins at 15% level of incorporation whereas deoiled rice bran protein concentrate can be incorporated at the level of incorporation.

CHAPTER V

SUMMARY

Rice (*Oryza sativa*) is one of the most staple diets for humans specially in Asian countries. In India, the annual production of rice is about 105 million tons. The huge amount of production results in a large amount of rice by-products. One of the major by-products is rice bran, which is accounted for 8% of milled rice (Shih *et al* 1999). Rice bran is a source of proteins, oils, nutrients and calories (Saunders 1990). The oil in rice bran is generally extracted and used as high quality cooking oil. During the oil processing, a large amount of bran nutrition is lost along with its by-products like deoiled rice bran and soap stock. Deoiled rice bran which contains high level of proteins has not been utilized to its full potential. Since the large quantity of deoiled rice bran is available at cheaper price, it is necessary to utilize this un-trapped source of nutrients.

Rice bran protein is superior to other cereals protein because of its high PER along with lysine and hypo-allergic properties (Wang *et al* 1999), which make it favorable for human consumption. Although the nutritional potential of rice bran protein has been recognized, at present rice bran protein concentrates and isolates are not commercially available due to lack of commercially feasible extraction method. The most common method for production of rice bran protein is by alkali hydrolysis method followed by acid precipitation. An alternative method that allowed the extraction of rice bran protein at neutral and slightly basic pH levels involved the utilization of enzymes (Ansharullah *et al* 1997). An optimized method for isolation of deoiled rice bran protein is necessary to formulate the strategies for its application in food products. As a potential food ingredient, deoiled rice bran protein will be subjected to various processing conditions leading to conformational and structural changes in the proteins (Adebiyi *et al* 2009). So, the development of value-added products from deoiled rice bran proteins require information on its characteristics. The present investigation was undertaken with the objective to optimize the extraction methods for deoiled rice bran proteins, to isolate the deoiled rice bran protein fractions and to utilize rice bran or protein concentrates in wheat based food products.

Protein concentrate was extracted using alkali extraction method and enzymatic extraction method. Optimization of protein extraction by alkali extraction method was done using Box-Behnken design of Response surface methodology. Different protein fractions were isolated from deoiled rice bran protein concentrate and characterized for their antioxidant activity. Deoiled rice bran and deoiled rice bran protein concentrate was incorporated in cookies at 10, 15 and 20 % & 1.0, 2.0 and 3.0% substitution level respectively and was compared with control for chemical composition as well as for sensory evaluation.

The developed cookies were analyzed for chemical composition by the method of (AOAC 2000). Deoiled rice bran was incorporated in muffins at 5, 10 band 15 % substitution level. The developed cookies and muffins were sensory evaluated for appearance, color, flavor, texture and overall acceptability by a panel of judges on a nine point hedonic scale. Rice bran contained 12.1 % moisture, 12.52 %, crude fiber and 14.92 % crude protein respectively. Ash content was 9.7% and silica content was 0.6% in deoiled rice bran. Optimum process variables for extraction of protein from DRB with alkali extraction method were 0.175 B/W ratio, 9.5 pH of solution and 45 min of extraction time which gave protein yield 8.63% and protein conc. 83.75%. Yield and protein content of protein concentrate were found to be affected by extraction conditions viz. pH, B/W ratio and time. pH had most significant effect on yield of protein concentrate followed by time of extraction and B/W of extraction solvent. Protein extracted with enzymatic method had better yield and quality of protein as compared to extraction with chemical method. Adding 0, 700, 1400, 2100 units of amylase to 100g deoiled rice ran resulted in 7.08%, 10.0%, 12.87%, 13.5% yield of protein respectively with 57.29%, 62.94%, 65.86% and 58.46% protein respectively. Addition of 0.9 unit to 2.7 units of enzyme protease resulted in gradual increase in yield of protein concentrate, however % protein in concentrate increase only with 1.8 unit of protease. Use of enzyme protease gave better yield as compared to use of amylase for protein extraction. Albumin(37.23%), globulin(20.27%) and glutelin(46.82%) are three major fractions of DRBP, while prolamin(1.18%) is a minor component. Highest anti-oxidative activity was detected in albumin fractions followed by glutelin, then globulin. The level of protein and fibre in cookies increased gradually with the increase in level of deoiled rice bran to incorporate.

In the sensory evaluation, the cookies with 1% level of incorporation of DRBP were most accepted, whereas DRB incorporated cookies at 10% level were most liked. In the sensory evaluation of muffins, 15% level of incorporation was most accepted by the panel of judges.

REFERENCES

Adebiyi A P, Adebiyi A O, Hasegawa Y, T Ogawa and Koji M (2009) Isolation and characterization of protein fractions from deoiled rice bran. *J Eur Fd Res Technol* **228**:391–401

Adebiyi A P and Aluko R E (2011) Functional properties of protein fractions obtained from commercial yellow field pea (Pisum sativum L) seed protein isolate. *J Fd Chem* **128**:902-8

Adebiyi A P, Adebiyi A O, J Yamashita, T Ogawa and K Muramoto (2009) Purification and characterization of antioxidative peptides derived from rice bran protein hydrolysates. *J Eur Fd Res Technol* **228**: 553-63.

Agboola S, Mills D (2005) Characterization and functional properties of Australian rice protein isolates. *J Cereal Sci* **41(3)**:283-90

Ali R, Shih F F and Riaz M N (2010) Processing and Functionality of Rice Bran Proteins and Peptides in Bioactives Proteins and Peptides as Functional Food and Nutriceuticals. IFT Press and Wiley-Blackwell. Chapter, 16.

Ansharullah, Hourigan J A and Chesterman C F (1997) Application of carbohydrases in extracting protein from rice bran. *J Sci Fd Agric* **74**: 141–46.

AOAC Official method of analysis (2000) 17th Edition. Association of official analytical chemists, Washington, DC.

Apinunjarupong S, Lapnirun S and Theerakulkait C (2009) Preparation and some functional properties of rice bran protein concentrate at different degree of hydrolysis using bromelain and alkaline extraction. *J Bio-Chem and Biotech* **39(2)**: 183-93

Barber S, Benedito-de, B C and Martenz, J (1981) Rice bran proteins: potential value of rice bran fractions as protein food ingredients. *Revista de Agroquimica y Tecnologia de Alimentos* **21**:247–56.

Box G E and Behnken D W (1960) Some new three level designs for the study of quantitative variables. Technometrics **2**: 455-75.

Bradford M M (1976) Arapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248-54.

Chagampang G B, Cruz L J, Espiritu S G, Santiago R G and Juliano B O (1966) Studies On The Extraction And Composition Of Rice Proteins *Cereal Chem*

Cheftel J C, Cuq J L and Lorient D (1985) Amino Acids, Peptides and Proteins. *Fd Chem*

Chiou T Y, Neoh T L, Kobayashi T and Adachi S (2012) Extraction of Defatted Rice Bran with Subcritical Aqueous Acetone. *Biosci Biotechnol Biochem* **76(8)**: 1535-39

Connor M A, Saunders R M and Kohler G O (1976) Rice Bran Protein Concentrates Obtained By Alkaline Extraction. *Cereal Chem* **53(4)**: 488-96

Cox G M and Cochran W G (1964) Experimental designs. New York: John Wiley & Sons, Inc.

De Groot A P and Slumps P (1969) Effect of severe alkali treatment of proteins on amino acid composition and nutritive value. *J Nutr* **98**:45–48.

Devi R R and Arumughan C (2007) Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. *J Bio res Technol* **98**: 3037–43

Esa N M, Ling T B, Peng L S (2013) By-products of Rice Processing: An Overview of Health Benefits and Applications. J Rice Res 1:107. doi: 10.4172/jrr.1000107

Graf E, Labuza T P and Empson K L (2006) Phytic acid as a food antioxidant. . *J Fd Sci* **56 :**560-63

Guan X and Yao H (2008) Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology. *J Fd Chem* **106**:345–51

Hamada J (1999) Use of protease to enhance solubilization of rice bran proteins. *J Fd Biochem* **23**:307-21.

Hamada J (2000) Characterization and functional properties of rice bran proteins modified by commercial exoproteases and endoproteases. *J Fd Sci* **65(2)**:305-10

Hamid A A and Luan Y S (2000) Functional properties of dietary fibre prepared from defatted rice bran. *Fd Chem* **68**: 15-19

Hanmoungjai P, Pyle D L and Niranjan K (2001) Enzymatic Process for Extracting Oil and Protein from Rice Bran. *J AOCS* **78**: 817-22

Hata S, Wiboonsirikul J, Maeda A, Kimura Y and Adachi S (2007) Extraction of defatted rice bran by subcritical water treatment. *J Biochem E ng* **40**: 44-53

Helm R M and Burks AW (1996) Hypoallergenicity of rice bran protein. *Cereal Foods World* **41(11):** 839-43.

Iqbal S, Bhanger M I, Anwar F (2005) Antioxidant properties and components of some commercially available available varieties of rice bran in Pakistan. *Fd Chem* **93**: 265-72

Jayadeep, Singh V, Rao V S, Srinivas A and Ali S Z (2009) Effect of physical processing of commercial De-oiled rice bran on particle size distribution, and content of chemical and bio-functional components. *J Fd Bioprocess Technol* **2**:57–67

Jiamyangyuen S, Srijesdaruk V and Harper W J (2005) Extraction of rice bran protein concentrate and its application in bread Songklanakarin. *J Sci Technol* **27(1):** 55-64

Jongjareorak A, Srikok K, Leksawasdi N and Andreotti C (2015) Extraction and Functional Properties of Protein from De-oiled Rice Bran. *J Nat Sci* **14(2)**: 163

Ju Z Y, Hettiarachchy N S and Rath N (2001) Extraction, Denaturation and Hydrophobic properties of rice flour proteins. *J Fd Sci* **66**: 229-32

Juliano B O (1985) Rice bran. In Rice: Chemistry and Technology; American Association of Cereal Chemists: St. Paul, MN.

Kahlon T S, Saunders R M, Chow F I, Chiu M M and Betschart A A(1990) Influence of rice bran, oat bran and wheat bran on cholesterol and triglycerides in hamsters. *J Cereal Chem* **67(5)**:439-43

Kinsella J E (1981) Functional properties of proteins: possible relationships between structure and function in foams. *Fd Chem* **7**:273-88.

Landers P S and Hamaker B R (1994) Antigenic properties of albumin, globulin and protein concentrate fractions from rice bran. *Cereal Chem* **71**: 409-11

Lloyd B J, Siebenmorgen T J and Beers K W (2000) Effects of Commercial Processing on Antioxidant in Rice Bran *Cereal Chem* **77(5)**:551-55

Losso J N, Bonsode R R and Bawadi H A(2003) Perfusion Chromatography Purification of 15 kDa Rice Prolamin. *J Agri Fd Chem* **51(24)**: 7122-26

Lowry O H, Rosebrough N J, Farr A L and Randall R J (1951) Protein measurement with the Folin-phenol reagents. *J Biol Chem* **193**: 265-75

Luh B S, Barber S and Benedito C de Barber (1991) Rice utilization vol. 2, Luh B S ed New York, NY: AVI Book Published by Van Nostrand Reinhold, pp:313-62

Mawal Y R, Mawal M R and Ranjekar P K (1990) Biochemical and immunological characterization of rice albumin. *Plant Sci* **70**:73-80

Montgomery D C (2004) Designs and analysis of experiments. New York: John Wiley & Sons.

Padhye V W and Salunkhe D K (1979) Extraction and Chararcterization of Rice Proteins. *Cereal Chem* **56(5)**: 389-93

Parrado J, Miramontes E, Jover M, Gutierrez J F, Teran L C and Bautista J (2006) Preparation of a rice bran enzymatic extract with potential use as functional food. *J Fd Chem* **98**: 742–48

Prakash J (1996) Rice bran proteins: Properties and food uses. Crit. Rev. *J Fd Sci Nutr* **36**: 537-52.

Prasad M N, Sanjay K R, Shravya K M, Vismaya M N, Nanjunda S S (2011) Health Benefits of Rice Bran - A Review. *J Nutr Fd Sci* 1:108. doi:10.4172/2155-9600.1000108

Rahim N I A, Morad N A and Long K (2015) Rice Bran Water Extraction through Autoclaving and Sonication: Protein Content and Amino Acid Profile. *J Appl Sci Agri* **10(5)**: 97-103

Rosniyana A, Hashifah M A and Noorin S A S (2009) Nutritional content and storage stability of stabilized rice bran- MR 220. *J Trop Agric and Fd Sci* **37(2):**163-70

Sathe S K and Venkatachalam M (2007) Val bean (Lablab purpureus L.) proteins: composition and biochemical properties. . *J Fd Sci* **87:** 1539-49

Satio K, Jin D H, Ogava T, Muramoto K, Hatekeyama E, Yasuhara T and Nokihara K (2003) Isolation and characterization of protein fractions from de-oiled rice bran. *J Agri Food Chem* **51**:3668-74

Saunders R M (1990) The properties of rice bran as a food stuff. *J Cereal Fd World* **35(7)**:632-36

Sekhon K S, Dhillon S S, Singh N and Singh B (1997) Functional suitability of commercially milled rice bran in india for use in different food products. *Plant foods for human nutrition* **50 :**127-40

Sereewatthanawut I, Prapintip S, Watchiraruji K, Goto M, Sasaki M and Shotipruk A (2007) Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *J Bioresour Technol* **99(3)**:555-61

Sharif M K, Butt M S, Anjum F M and Nawaz H (2009) Preparation of Fiber and Mineral Enriched Defatted Rice Bran Supplemented Cookies. *Pak J Nutri* **8 (5):** 571-77

Shih F F and Diagle K W (2000) Preparation and Characterization of Rice Protein Isolates. *JAOCS* **77**: 885-89

Shih F F, Champagne E T, Daigle K and Zarins Z (1999) Use of enzymes in the processing of protein products from rice bran and rice flour. *Nahrung* **43**: 14–18

Tang S, Hettiarachchy N S and Shellhammer T H (2001) Protein extraction from heat-stabilized defatted rice bran. I. Physical processing and enzyme treatments. *J Agri Fd Chem* **50**: 7444- 48.

Tang S, Hettiarachchy N S, Horax R and Eswaranandam S (2003) Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. *J Fd Sci* **68 (1):**152–57

Theerakulkait C, Chaiseri S and Mongkolkanchanasiri S (2006) Extraction and Some Functional Properties of Protein Extract from Rice Bran. *J Nat Sci* **40**:209-14

USDA, PSD Online. June 10, 2009

Wang M, Hettiarachchy N S, Qi M, Burks W and Siebenmorgen T (1999) Preparation and Functional Properties of Rice Bran Protein Isolate. *J Agri Fd Chem* **47**: 411-16

Xia N, Wang J, Yang X, Yin S, Qi J, Hu L and Zhou X (2012) Preparation and characterization of protein from heat-stabilized rice bran using hydrothermal cooking combined with amylase pretreatment. *J Fd Eng* **110(1):**95-101

Yadav R B, Yadav B S and Chaudhary D (2011) Extraction, characterization and utilization of rice bran protein concentrate for biscuit making. *British Fd J* **113(9):**1173 – 82

Zhang H J, Zhang H, Wang L, Guo X N (2012) Preparation and functional properties of rice bran proteins from heat-stabilized defatted rice bran. *J Food Research Int* **47**: 359–63

VITA

Name of the student	**:**	Gurleen Singh Mann
Father's name	**:**	S. Davinder Singh Mann
Mother's name	**:**	Smt. Daljit Kaur
Nationality	**:**	Indian
Date of birth	**:**	4th June, 1992
Permanent home address	**:**	Village Doraha, Tehsil Payal District Ludhiana – 141421, Punjab

EDUCATIONAL QUALIFICATIOIN

Master's degree	**:**	Integrated Master of Science (Hons.) in Biochemistry
Minor subject	**:**	Food Science and Technology
University and year of award	**:**	Punjab Agricultural University, Ludhiana 2016
OCPA	**:**	6.70/10.00
Title of Master's thesis	**:**	Protein quality of deoiled rice bran and its use in food products