In The Name of Allah The Most Merciful The Most Compassionate And The Most Beneficent
BIOCHEMICAL AND TECHNOLOGICAL EVALUATION OF RICE BRAN FOR THE PRODUCTION OF HIGH NUTRITIVE BAKERY PRODUCTS

By

MUMTAZ SHAHEEN

B.Sc. (Hons.) Agri. (UAF)
M.Sc.(Hons.) Food Tech. (UAF)

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
FOOD TECHNOLOGY

INSTITUTE OF FOOD SCIENCE & TECHNOLOGY
UNIVERSITY OF AGRICULTURE
FAISALABAD
2004
The Controller of Examinations,
University of Agriculture,
Faisalabad.

We, the members of the supervisory committee, certify that the contents and form of dissertation submitted by Mr. Mumtaz Shaheen, Regd. No. 79-ag-1008 have been found satisfactory and recommend that it be processed for evaluation by the External Examiner(s) for the award of the degree.

SUPERVISORY COMMITTEE

I. Chairman

Dr. Faqir Muhammad Anjum

II. Co-supervisor

Dr. Allah Ditta Khan

III. Member

Dr. Tahir Zahoor

IV. Member

Dr. Must. Ahmad Shiekh
DEDICATED

TO

HOLY PROPHET HAZRAT MUHAMMAD

(Peace Be Upon Him)
ACKNOWLEDGEMENTS

I am highly indebted to Allah Almighty who blessed to conduct this research work presented in this dissertation. I offer my humbly thanks from the deep sense of heart to the Holy Prophet Hazrat Muhammad (Peace Be Upon Him), the educator of human race, that without him the life would have been meaningless.

I feel great pleasure and honour to express my gratitude to my supervisor, Dr. Faqir Muhammad Anjum, Professor and Director, Institute of Food Science & Technology, University of Agriculture, Faisalabad for his valuable suggestions, scholarly guidance and kind help for execution of my research and completion of this dissertation.

It is profound privilege to express my appreciation to Dr. Allah Ditta Khan, Chief Scientific Officer, PCSIR Labs. Complex, Lahore for the accomplishment of the research work presented in this manuscript.

I am indebted to my committee members, Dr. Tahir Zahoor, Associate Professor, Institute of Food Science & Technology and Dr. Munir Ahmad Sheikh, Professor and Chairman, Department of Chemistry for their sympathetic attitude and kind cooperation during the conduct of this study.

I also wish to pay my sincerest thanks to Dr. Masood Sadiq Butt, Associate Professor, Institute of Food Science & Technology, who helped me for final presentation of this exposition.

The author is deeply grateful to Mr. Javed Islam Agha, Resident Director, Reem Rice Mills Private Limited, Muridke, Sheikhupura for providing incentive and opportunity during the course of this study.

Furthermore, I express my sincere and special gratitude to Mr. Anwaar Ahmed Ph.D scholar for his kind cooperation in writing this manuscript.

Finally, I would like to express my sincere gratitude to my parents, wife and son for their wishful prayers.

Mumtaz Shaheen
## CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>REVIEW OF LITERATURE</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>MATERIALS AND METHODS</td>
<td>31</td>
</tr>
<tr>
<td>IV</td>
<td>RESULTS AND DISCUSSION</td>
<td>45</td>
</tr>
<tr>
<td>V</td>
<td>SUMMARY</td>
<td>130</td>
</tr>
<tr>
<td>B</td>
<td>LITERATURE CITED</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>APPENDICES</td>
<td>157</td>
</tr>
<tr>
<td>Table</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Treatments for the preparation of bakery products from wheat flour and processed rice bran</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>Composition of various diets containing chapatis</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Composition of experimental rations for maize substitution with rice bran</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>Composition of experimental rations for wheat substitution with rice bran</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>Mean squares for proximate composition of processed rice brans</td>
<td>47</td>
</tr>
<tr>
<td>6</td>
<td>Means for proximate composition of processed rice bran</td>
<td>48</td>
</tr>
<tr>
<td>7</td>
<td>Mean squares for chemical composition of processed rice brans</td>
<td>50</td>
</tr>
<tr>
<td>8a</td>
<td>Means for proximate chemical composition of processed rice bran</td>
<td>51</td>
</tr>
<tr>
<td>8b</td>
<td>Means for haemagglutinin-lectin, trypsin inhibitor activity and phytates of raw and processed rice bran</td>
<td>59</td>
</tr>
<tr>
<td>9</td>
<td>Bacterial count, mould count, color and structure after 90 days of raw and processed rice bran</td>
<td>64</td>
</tr>
<tr>
<td>10</td>
<td>Mean squares for chemical composition of various processed rice bran supplemented breads</td>
<td>66</td>
</tr>
<tr>
<td>11</td>
<td>Chemical composition of various processed rice bran supplemented breads</td>
<td>67</td>
</tr>
<tr>
<td>12</td>
<td>Mean squares for external characteristics of various processed rice bran supplemented breads</td>
<td>71</td>
</tr>
<tr>
<td>13</td>
<td>Means for external characteristics of various processed rice bran supplemented breads</td>
<td>72</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Means squares for internal characteristics of various processed rice bran supplemented breads</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Means for internal characteristics of various processed rice bran supplemented breads</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Mean squares for chemical composition of processed rice bran supplemented wheat flour cookies</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Means for chemical composition of processed rice bran supplemented wheat flour cookies</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Mean squares for sensory evaluation of various processed rice bran supplemented wheat flour cookies</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Mean for sensory evaluation of various processed rice bran supplemented wheat flour cookies</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Mean squares for chemical composition of chapatis of wheat flour supplemented with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Means for chemical composition of chapatis of wheat flour supplemented with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Mean squares for various sensory attributes of chapatis of wheat flour supplemented with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Means for various sensory attributes of chapatis of wheat flour supplemented with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Mean squares for various nutritional attributes in different groups of albino rats fed on various experimental diets prepared from various PRB supplemented chapatis</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Means for various nutritional attributes in different groups of albino rats fed on various experimental diets prepared from various PRB supplemented chapatis</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Mean squares for performance of broiler chicks fed on diets containing maize replaced with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Performance of broiler chicks fed on diets containing maize replaced with processed rice bran</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Mean squares for feed cost of broiler chicks fed on diets containing maize replaced with processed rice bran</td>
<td></td>
</tr>
</tbody>
</table>
29 Means for feed cost of broiler chicks fed on diets containing maize replaced with processed rice bran 117
30 Mean squares for performance of broiler chicks fed on diets containing wheat replaced with processed rice bran 120
31 Means for performance of broiler chicks fed on diets containing wheat replaced with processed rice bran 121
32 Mean squares for feed cost of broiler chicks fed on diets containing wheat replaced with processed rice bran 126
33 Means for feed cost of broiler chicks fed on diets containing wheat replaced with processed rice bran 127
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Free fatty acids of rice bran</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Effect of processing on peroxidase activity of rice bran</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>Effect of storage on bacterial count of rice bran</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>Effect of storage on mould count of rice bran</td>
<td>63</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Composition of vitamin premix</td>
<td>157</td>
</tr>
<tr>
<td>II</td>
<td>Composition of mineral premix</td>
<td>158</td>
</tr>
</tbody>
</table>
CHAPTER-I

INTRODUCTION

Pakistan is situated in the region, where 90% of the world’s rice is produced as the second biggest cash crop. It is the staple food for half of the world’s population and provides dietary energy and protein up to 75% for 2.5 billion people (Juliano, 1990). Rice bran a by-product of rice milling industry is an indispensable, soft and fluffy off-white powdery material abundantly available during the milling season, comprising of 10% by weight of the paddy (Saunders, 1990). This plentiful material is derived from the outer layers of the rice caryopsis and consists of fine particles of pericarp + seed coat + nucellus + aleuraone layer + embryo and part of sub-aleurone layer of the starchy endosperm, obtained from the polishing of brown rice (Juliano, 1988). The potential of producing rice bran at the global level is 27.3 million tons (Prakash, 1996). In Pakistan annual production of rice is 4500 to 5000 thousand tons and thus 450 to 500 thousand tons of rice bran is generated (GOP, 2004).

Rice bran is a good source of protein, lipids, dietary fiber, vitamins and minerals. Chemically it contains protein 11-17%, fat 11-18 %, fiber 10%, ash 9% and nitrogen free extract(NFE) 45-65%. It is a rich source of B- vitamins and minerals such as phosphorus, potassium, iron, copper and zinc. The amino acid profile of rice bran has been generally reported to be superior to cereal grain proteins (Farrell, 1994). The protein of rice bran is relatively of high nutritional value. As far as its nutrition is concerned, the protein efficiency ratio (PER) of rice bran has been reported to be in the range of 1.6 to 1.9 as compared to casein value of 2.5 while for its protein concentrates PER values ranged from 2.0 to 2.5. The digestibility of rice bran protein has been found to be 73% as compared to 90% digestibility.
denoted for its protein concentrate. It is a good source of lysine and methionine and can be an effective tool to supplement the lysine and methionine deficient foods such as in wheat, maize and sorghum to overcome the malnutrition problem prevailing among masses (Dale, 1997).

The rice bran is also thrilling for its good quality oil. The low contents of saturated fatty acids and high content of linoleic acid, poly-unsaturated fatty acids plus tocopherol make the rice bran oil as health beneficial food (Cornelius, 1980). It contains 75% unsaturated fatty acids in the form of oleic acid, linoleic acid and linolenic acid in proportion of 40:34:1, respectively. The fatty acid profile of rice bran is the closest to the American Heart Association and World Health Organization as compared to other edible oils. In Japan, it is known as “Heart oil”. The tocopherols and ferulic acid esters are potent anti-oxidants present in rice bran (Saunders, 1990). Stabilized full fat rice bran has hypocholesterolemic effect in humans, monkeys and rats (Hegsted et al., 1990; Nicolosi et al., 1989).

The major carbohydrates of rice bran are cellulose and hemicelluloses, however, the starch is also additionally present due to the breakage of endosperm during milling. The quantity of starch varies according to the amount of breakage and degree of milling, but values of 10-20% or even higher could be expected (Saunders, 1986). The rice bran has laxative effect with increments of increased faecal out put and stool frequencies (Tomlin and Read, 1988).

Undoubtedly, rice is one of the world’s basic food items but rice bran a valuable by-product of rice milling has yet not been efficiently utilized for human consumption. Despite of its excellent nutrition, hypoallergenic and recently claimed nutraceutical properties, rice bran is mainly used for poultry or animal feed as low quality ingredient. This situation demands that this healthy food ingredient may be incorporated in the daily diet.
However, the anti-nutritional or toxic factors present in rice bran limit potential as a food or feed ingredient. These factors include lipases, trypsin inhibitors, haemagglutinin-lectin and phytates etc. termed as phytotoxicins which get accumulated in bran during polishing of rice, hinder and mask the digestibility and availability of nutrients. Therefore it is used in livestock or poultry feed as low quality ingredient (Gunawan and Tangendjaja, 1988; Warren and Farrell, 1990). After milling the lipases and lipoxygenases when in contact with rice bran fat become active and rapidly (1% per hour) hydrolyzes into fatty acids which are further oxidized by atmospheric oxygen that are the key factors leading towards deterioration in the quality of oil. The microbial lipases and proteases also deteriorate the nutritional quality (Luh et al., 1991).

Trypsin inhibitors are peptides, which form complex with pancreatic enzymes i.e. trypsin, chymotrypsin and induce pancreatic hypertrophy. One mole of rice bran inhibitor can combine with two moles of trypsin (Yokochi, 1972). Its activity (85-95%) was found in the embryo. The pancreatic hypertrophy induced by trypsin inhibitor has been reported in poultry fed raw rice bran (Barber et al., 1978). Phytic acid as phytates inhibits the digestibility and availability of nutrients in rice bran by formation of complexes with minerals, protein, digestive enzymes and amino acids particularly lysine, methionine, arginine and histidine (Jangbloed et al., 1991). Haemagglutinin-lectin, a toxic glycoprotein is present in the rice bran, which agglutinates mammalian red blood cells metabolites (Benedito and Barber, 1978). Moreover, the field fungi and bacteria present in rice bran also produce lipases, proteases and amylases, which destroy nutrients, produce off-flavors, impart browning of color, give bitter taste and even produce aflatoxins (Schroeder, 1969).

The literature indicated that except “phytates” all the other undesirable factors present in rice bran are protein in nature. Therefore it
may be postulated that mild acid or alkali treatment with thermal cooking can denature or modify the structure of these toxic enzymes, proteins and hydrolyze phytates into inositoi and phosphoric acid. Moreover, these chemical and physical treatments may augment the availability of minerals like iron, calcium, zinc and particularly phosphorus. Therefore in order to utilize rice bran nutritional potential efficiently, these anti-nutritional or toxic factors must be eliminated or minimized to improve its nutritional quality. Although many efforts have been made in the past but generally focused to remove one or the other toxic factor only.

Recently Sharma and Chauhan (2002) stabilized rice bran by using dry heat and extrusion cooking and prepared bread and cookies by supplementing the stabilized rice bran in wheat flour at levels of 5 to 20%. Rice bran supplementation in wheat flour improved the proteins, fat, lysine and dietary fiber contents in bread and cookies proportionately to the level of supplementation. Stabilized full fat rice bran up to 20% level and un-stabilized full fat or stabilized defatted rice bran up to 10% have been found to be suitable in various food products (Singh et al., 1995). Rice bran was supplemented with wheat flour at 15 to 30% in yeast bread and it was concluded that rice bran can be incorporated successfully up to 15% replacement level without affecting loaf weight, height or volume (Sharp and Kitchen, 1990). Defatted rice bran increases dough yield, contributes to an attractive crumb & crust, does not disturb fermentation or mixing tolerance of dough, causes baked products to remain fresh, moist and adds significant amino acids, minerals and vitamins to baked goods (Lynn, 1969). Owing to its composition and apparent hypoallergenicity, rice bran has many applications in a diet, which may be characterized by high in dietary fiber and low in saturated fat. It may be particularly beneficial to those individuals who are allergic to other cereal grains. However, strong evidences are also available that the consumption of rice bran may be
beneficial in reducing the risk of cardiovascular disease and colon cancer (Marshall and Wadsworth, 1994).

The advancement in recent comprehensions and skills in food system have made it possible to remove undesirable anti-nutritional factors in an efficient and economical way. The preparations of various diversified food products after improving and up grading the nutritional quality of rice bran have been possible by combining the various appropriate techniques. To test this hypothesis, the present study was therefore, carried out to develop a system to denature all the toxic factors i.e. lipase, peroxidase, haemagglutinin-lectin, trypsin inhibitor and to hydrolyze phytates in a single operation using combined chemical and physical treatments.

Therefore, the present research project has been taken up to achieve the following objectives.

i) Removal of anti-nutritional factors present in rice bran through chemical and physical means.

ii) Chemical evaluation of rice bran and its supplemented bakery products.

iii) To find out the effect of rice bran supplemented wheat flour samples on the quality of bread, cookies and chapaties.

iv) Biological assay of rice bran supplemented bakery products.

v) To develop an appropriate and economical technique for its commercialization in food products.
Chapter-II

**REVIEW OF LITERATURE**

Rice bran a by-product of rice milling industry is a soft and fluffy powdery material composed of several botanical entities i.e. pericarp, seed coat, nucellus, aleurone layer, germ and part of subaleurone layer of the starchy endosperm. A variable quantity of the subaleurone of endospermic material is normally present in the bran fraction, depending upon the severity of milling. Breakage of the white kernel during milling also results in small fragments of the endosperm becoming part of bran fraction. These broken fragments are primarily starch and normally comprise 10-20% of the rice bran (Saunders, 1990). The amount of starch and other nutrients in the bran depends on the degree of milling and extent of endosperm breakage. The bran is a rich source of valuable nutrients. Owing to its overall composition, nutritional profile and functional characteristics it has many food applications especially in bakery products like bread, cookies and chapattis.

The literature available pertaining to various aspects of the present study has been reviewed under the following headings:

1. Importance of Rice Bran
2. Chemical Composition of Rice Bran
3. Anti-nutritional Factors
4. Processing of Rice Bran
5. Physico-Chemical Characteristics of Wheat Flour
6. Supplementation of Rice Bran for Bakery Products
7. Biological Assay
2.1 Importance of Rice Bran

Rice bran is a less expensive and abundantly (₹ Rs.5-7/kg) available as soft fluffy by-product of rice milling industry in the Asian region where 90% of world’s rice is produced as the second biggest agricultural food crop. Its seasonal production ranges between 0.45-0.50 million tons in Pakistan (GOP, 2004), however nutrient density, amino acid and fatty acid profiles are superior to cereal grains. Moreover nutritional and functional properties of rice bran are well suited for bakery products. Its consumption is beneficial in reducing the risk of colon cancer and cardiovascular disease (Marshall and Wadsworth, 1994).

Rice bran contains at least 78% of the rice kernel’s thiamin, 47% of the riboflavin and 67% of the niacin. It is also rich in other B-complex vitamins and good source of minerals too i.e. calcium, magnesium, zinc, phosphorus and manganese etc. Moreover it contains about 80% of the rice kernel iron (Saunders, 1990).

Rice bran oil is a miracle product obtained from the outer brown layer of rice germ. Generally the rice bran contains 15-20% oil (Marshall and Wadsworth, 1994). It is extensively used in Japan, Korea, China, Taiwan and Thailand as premium edible oil. In Japan, it is more popularly known as “Heart Oil”. In western countries, it has acquired the status of a “Health Food”. It is an excellent cooking medium because of nutritional superiority, abundant micronutrients, longer shelf life as well as stability at higher temperature, better taste and flavor to food items. Frying takes less time but ultimately saves energy and economic benefit due to 15% less absorption of oil during frying (Sharma, 2002).

Lloyd et al. (2000) reported that rice bran contained high amount of beneficial anti-oxidants including tocopherols, tocotrienols and oryzanols. Milling technology produced rice bran from different layers, which were blended, and then steam extruded to form a stabilized rice bran. The rice
bran intermediates varied in anti-ioxidant content. They investigated the changes in anti-oxidants in rice bran from both long and medium grain rice during commercial milling and bran processing. Bran collected after milling break II had the highest level of tocopherol and tocotrienol. Oryzanol concentration was significantly higher in outer bran layers. The results also indicated that the long grain rice bran provided approximately 15% more anti-oxidants than the medium grain rice bran.

Zhimin et al. (2001) explored the anti-oxidant activities of vitamin E and gamma-oryzanol components form rice bran in order to improve the understanding of the mechanism underlying the physiological effects of rice bran on cholesterol metabolism. Activities of vitamin E (alpha-tocopherol, alpha-tocotrienol, gamma-tocopherol and gamma-tocotrienol) and gamma-oryzanol components purified from rice bran were studied in a cholesterol oxidation system. The quantity of gamma-oryzanol is up to 10x higher than that of vitamin E in rice bran. It was suggested that gamma-oryzanol may be more important anti-oxidant for the reduction of cholesterol than vitamin E, which has been considered previously to be the major anti-oxidant.

Kahlon et al. (1990) reported that rice bran lowered serum cholesterol and liver cholesterol level in hypercholesterolemic hamsters to the same extent as did oat bran, possibly through increasing the excretion of fat and neutral sterols.

Stabilized full fat rice bran or oil has hypocholesterolemic effect in humans, monkeys and rats (Gerhardt and Gallo, 1989; Hegsted et al., 1990). Rice bran fiber has laxative effect with increased faecal outlet and stool frequencies (Tomlin and Read, 1988).

Kahlon et al. (1994) reviewed cholesterol lowering effects of rice bran with reference to studies carried out on hamsters, rats, other animal species and humans. Results demonstrated a cholesterol lowering effect
with rice bran in hypercholesterolemic individuals, particularly reduction occurred in the low density lipoprotein. Possible mechanisms leading to cholesterol reduction by rice bran and the benefits of inclusion rice bran in the diet were considered. Specific rice bran components showed hypocholesterolemic activity i.e. rice bran oil, however intact full fat rice bran appears to be most effective.

2.2 Chemical Composition of Rice Bran

Rice bran is potentially untapped source of nutrients and a sustainable food ingredient. The composition of rice bran depends upon many factors associated with the rice grain itself and the milling process. Major factors associated with rice bran are varietal and environmental variability in average chemical composition, distribution of chemical constituents, thickness of anatomical outer layers, size and shape of grains and hardness or resistance of grains to breakage and abrasion (Houston, 1972).

Mahtab (1985) concluded that the extent of variability in nutrient composition of rice bran depended on milling process and storage conditions. The composition also varied due to varieties and processing conditions (Rao and Reddy, 1986).

The by-products obtained during first to final stage produced rice polishing had dry matter 91.57 to 90.89%, nitrogen 2.75 to 2.52% and crude fiber 9.31 to 6.13%, respectively. On the basis of composition it was suggested that different rice by-products obtained from various processing steps could be used as a mixture for further uses (Garcia and Figueroa, 1989).

Warren and Farrell (1990) analyzed nutritive value of rice bran of several cultivars grown in Australia and reported that the chemical composition of all the cultivars were reasonably uniform. The crude protein content ranged from 13.4-17.4%, ether extract 20.4-23.4%, neutral
detergent fiber was found to be 25.6%, acid detergent fiber 12.2% and ash content 10.5%.

Rice bran contains protein 11-17%, fat 11-18%, fiber 10%, ash 9% and NFE 45-65% as well as vitamins. Its chemical profile is comparable to other cereals like wheat, maize and sorghum. It is also a rich source of minerals like phosphorus, potassium, iron, copper and zinc. The amino acid profile of the rice bran protein is generally superior to that of cereal grains (Farrell, 1994).

Malik and Chughtai (1979) observed the chemical composition of rice bran i.e. crude protein 11.54%, ether extract 14.65% and crude fiber 3.85%. However, Gohl (1981) determined protein content ranging from 11.8-13.0%, ether extract 10.1-12.4%, crude fiber 2.3-3.2% and ash 5.2-7.3%. Later on Farrell (1994) observed that fiber content ranged from 10 to 15%.

Rice bran has been reported to be rich in oil of good quality. The high content of unsaturated fatty acids (74%) and tocopherol make it as health beneficial food (Cornelius, 1980). It contains 75% unsaturated fatty acids i.e. oleic acid (C18:1=40), linoleic acid (C18:2=34) and linolenic acid (C18:3=1), moreover the tocopherols and ferulic acid esters are the potent anti-oxidants present in rice bran as reported by Saunders (1990).

Al-Jassar and Al-Mustafa (1996) analyzed quality of Hassawi rice bran. The bran was ground and proximate analysis was conducted. The extracted oil was characterized and fatty acids were determined. The results showed normal level of protein and fat were 12.56 and 15.15%, respectively. The high level of the unsaturated fatty acid i.e.linoleic acid indicates the good nutritional quality of the oil. The mineral content of the rice bran revealed the presence of high potassium, calcium and magnesium. Furthermore, it contains an appreciable amount of iron. It is recommended that more studies be carried out on the addition of a small
amount of rice bran to bread flour to enrich the bread with the above nutrients and to raise its nutritional value.

Pomeranz and Oryl (1982) gave the range of proximate composition of rice bran from 13.2-17.3% proteins, 9.5-13.2% crude fiber and 9.2-11.2% ash on dry weight basis.

Farinu et al. (1989) analyzed nutrient composition, carbohydrate fraction and amino acid content in 56 rice bran samples and 5 groups of rice milling by-products. Rice bran contained crude protein 73, crude fiber 290 and NFE 404 g/Kg dry matter. Crude protein contents in milling by-products were 7 % (rough bran), 13-14 % (soft bran) and 15-17 % (broken rice) and crude fiber 27, 17-19 and 4-5%, respectively. Total soluble carbohydrates were 10, 18 and 78-87% and cell wall constituents were 67, 47-55 and 11-18% for rough bran, soft bran and broken rice, respectively. Amino acid contents were similar in all rice by-products. Energy values increasing order were rough bran, soft bran and broken rice.

The major carbohydrates in rice bran are cellulose (9.6-12.8%) and hemicelluloses (8.7-11.4%). The starch is also get included due to breakage of endosperm during milling. The quantity of starch varies according to the amount of breakage and degree of milling but it ranged from 10-20% however sugar 3-8% could be expected (Saunders, 1990).

In the most recent studies, Bhanja and Verma (2001) analyzed defatted rice bran obtained from two types of extraction processes i.e. Batch and Continuous. They analyzed crude protein, ether extract, total ash, calcium, phosphorus, glucose and starch content of defatted rice brans (batch and continuous) were found to be 19.00%, 0.79%, 17.05%, 0.11%, 1.92%, 2.30%, 11.22% and 15.02%, 1.56%, 13.00%, 0.40%, 2.76%, 2.16%, 19.00%, respectively.
2.3 Anti-nutritional Factors

2.3.1 Lipases

Lipases are enzymes present naturally in paddy, separately housed from fat. In milling these are in contact with rice bran fat. Rice bran contains 13-20% fat out of which 74% contains unsaturated fatty acids. Fat in paddy is quite stable because of separate compartmentation of oil and enzymes but due to milling, the lipases and lipo-oxygenases become active and rapidly hydrolyze (1% per hour) the unsaturated fat into fatty acids, which are oxidized by atmospheric oxygen (Desikacheri, 1974). The microbial lipases and proteases also deteriorate the nutritional quality. The rancid oil and their related products are toxic therefore are considered anti nutritional factors.

Rice bran contains 12 - 22% oil with a fairly high amount of palmitic, oleic and linoleic acids making up more than 90% of the total fatty acids and out of which 74% are unsaturated fatty acids (Saunders, 1990). Once paddy is milled, there is an immediate and rapid hydrolytic release of fatty acids, which further breakdown by the action of lipoxygenases present in rice embryo (Shaheen et al., 1975; Ryu and Cheigh, 1980; Farrell, 1994). The rate of hydrolysis is 1% per hour and about 70% of fat is hydrolyzed in a month (Yokochi, 1977). The hydrolysis increases with temperature and humidity. The hydrolytic rancidity severely affects the nutritive value and palatability of rice bran (Tsai, 1982). Digestive disorders have been reported when rice bran containing deteriorated oil was fed to the chicken (Yokochi, 1972; Subrahmanyan, 1977). Similarly, depressed chicken growth and declined metabolized energy were observed when rancid rice bran was fed to chicken (Gunanwan and Tangendjaja, 1988).

Rajeshwara and Prakash (1995) examined inactivation of rice bran lipase by various agents. Apparent thermal denaturation temperature of treated enzyme indicated that its active site was buried deep inside the
molecule and was only accessible following an unfolding stage, which could be followed by an inactivation procedure.

2.3.2 Trypsin Inhibitors

Trypsin inhibitors are also endogenous enzymes chemically peptides, which can form stable and inactive complex with proteolytic pancreatic enzymes i.e. trypsin and chemotrypsin. Due to complex formation, the activity of these enzymes decreases and poultry performance is reduced. Rice bran contained trypsin inhibitor (Kratzer and Payne, 1977; Deolankar and Singh, 1979). The trypsin inhibitors isolated from rice bran are rich in basic amino acids. One mole of rice bran trypsin inhibitor can inhibit two moles of trypsin. It is stable at acidic or neutral pH and heat (90°C) resistant. Approximately 85-95% trypsin inhibitor activity was found in rice embryo. The inhibitor induces pancreatic hypertrophy in poultry fed raw rice bran (Barber et al., 1978).

Kratzer et al. (1974) reported improved growth response and feed efficiency in chicks by extraction of anti-trypsin activity with 1 percent dilute acetic acid or denaturation by autoclaving or steaming of rice bran. They also observed that pancreatic hypertrophy in chicks correlated with anti-trypsin activity in rice bran since pancreatic secretion was controlled by feedback inhibition mechanism based on level of trypsin in the intestinal tract.

2.3.3 Phytate

Phytic acid as phytates of essential minerals i.e. phosphorus, zinc and manganese etc. exist up to 4-6% (Nelson et al., 1968). The phytates block the digestibility and availability of nutrients in rice bran by formation of complexes with minerals, protein, digestive enzymes and amino acids particularly lysine, methionine, arginine and histidine (Jangbloed et al., 1991).
It is a rich source of minerals particularly phosphorous, zinc and ferrous (Farrell, 1994). The principal storage form of these minerals in plants, particularly in cereals and their by-products are in the form of phytic acid (Gill, 1999).

Phytic acid is present in the form of phytin. It is complexed with protein, vitamin and minerals and reduces the bioavailability of these nutrients in poultry (Zyla et al., 1989; Bird, 1998).

Phytic acid commonly called myo-inositol or scientifically 1, 2, 3, 4, 5, 6 hexa (dihydrogen phosphate) and its salts i.e. phytates are found in many cereal grains and oil seeds (Kunckles et al., 1985). Phytic acid reduced body growth and deposition of Zn, Fe and Mn in tibias and it was found to be heat stable. It showed strong chelating properties due to its structure (Kanaya et al., 1976; Thompson and Weber, 1981; Ramzan, 2000).

Phytate constitutes 1-2% by weight of many cereals (Torre et al., 1991). Anjum et al. (2002) determined 2.23% phytic acid and 52.50 ppm iron in whole wheat flour of Pakistani wheats. The reduction in iron and phytic acid content has also been observed due to humid and hot condition persisted during storage or reactions of iron with other food components such as proteins, phytic acid and carbohydrates (Hinnai et al., 2000).

2.3.4 Haemagglutinin-lectin

Haemagglutinin-lectin are toxic proteins present in the rice bran, which agglutinate mammalian red blood cells metabolites. (Benedito and Barber, 1978). Haemagglutinins are globulin protein present in rice bran and agglutinate red blood cells (Tsuda, 1979; Ory et al., 1981). Similarly, lectin is a glycoprotein and is present in rice germs. It contains 27% carbohydrate, predominantly glucose (Takahashi et al., 1973) while another 10% carbohydrate is mainly in the form of xylose and arabinose.
The lectin also contains a large number of glycine and cystine residues (Tsuda, 1979). Some researchers (Tan et al., 1983) had reported variation in lectin content within a single species of plant and thus giving variable effects on red blood cells agglutination.

2.4 Processing of Rice Bran

The processing of rice bran can be carried out to inactivate endogenous lipases, hydrolyze phytic acid into myo-inositol and available phosphorus, denature the trypsin inhibitor and haemagglutinin-lectin proteins in such a way that their toxicity is destroyed without damaging the protein quality of rice bran. Furthermore it also destroyed the field fungi, bacteria and insects infestation, so that the bran becomes safe from further deterioration which alternately enhanced its shelf life.

Jamuna and Ramanatham (1995) prepared protein concentrates from unstabilized, acid stabilized, heat stabilized and parboiled rice bran in order to determine the effect of the different stabilization treatments. The stabilization treatments and techniques for drying protein concentrates did not alter amino acid profiles to a large extent. Essential amino acid profile, available lysine content and in vitro digestibility studies showed that rice bran protein is of good nutritional quality.

Saunders et al. (1982) reported that heat stabilized or acetic acid 1% extracted rice bran could be used at higher level in poultry diets.

Jiaxun (2001) described a method of stabilizing parboiled rice bran by acid treatment. An edible acid having anti-oxidative properties was added to parboiled rice bran to maintain the stability of the bran for at least 6 months at ambient conditions. Furthermore, this process was adopted to stabilize parboiled rice bran used in foods by adding 0.1-2% mild acetic acid to a parboiled rice bran food product, resulting in the stability of the food being maintained for at least 6 months at ambient conditions.
Purosothaman et al. (1989) treated defatted rice bran with 0.34 N hydrochloric acid and heated for 30 minutes. The chemical analysis revealed reduction in hemicellulose and cellulose by 47.9 and 36%, respectively and increased available carbohydrates about 30%.

Chaudhry and Miller (1998) tested the effect of calcium oxide, sodium hydroxide and NaOH plus hydrogen peroxide on cell wall composition, digestion and fermentation of wheat straw. All treatments improved the nutritive value of straw compared with untreated through modification of cell wall with a subsequent increase in digestibility. Although the digestibility for Ca was lower than that for Na despite reduction in cell wall, its use to treat straw was more safe and cost effective than Na.

Kratzer and Payne (1977) observed that autoclaving rice bran for 3-25 minutes significantly improved its feeding value as measured by growth rate of chicks. They further reported that autoclaving or parboiling destroyed the lipase activity in rice bran.

Purosothaman et al. (1990) studied the effect of autoclaving (120°C, 15 psi for 3 min) on the feeding value of deoiled rice bran. Analysis revealed decreased cell wall constituents (hemicellulose by 43.9% and cellulose by 9.9%) and increase total cell contents (by 28.1%) and available carbohydrates, glucose (by 11.3%) and starch (by 12.4%).

Yaqub et al. (1990) carried out the stabilization of rice bran in extrusion stabilizer. The result showed that free fatty acid (FFA)% of untreated (raw) and treated (stabilized) rice bran were 29.0 and 4.6%, respectively after 5 hours storage however, percentage of FFA were increased to 10.2 and 21.6%, respectively after 20-45 hours. This indicated that stabilization is an essential step to control FFA and store bran to reasonable period. The result also showed that increase of FFA depends upon time factor even after stabilization.
Maurice (1991) stated that extrusion cooking of rice bran converted a powdery dusty material with poor shelf life into a non dusty shelf stable product fit for direct utilization as a feed ingredient in feed formulation. There is a disadvantage with this method that the moisture is elevated during processing required a subsequent drying step. There are offsetting advantages in that, extremely long shelf life is possible with an expander cooker for feed applications. He further stated that processing through an expander cooker at elevated moisture levels followed by subsequent drying can effectively destroyed the activities of “Lipases” and produce a shelf stable, full fat rice bran.

Takemasa and Hijikuro (1991) reported that autoclaving defatted rice bran markedly decreased its phytate content. About half of the phytate phosphorus was hydrolyzed by autoclaving for 3-5 hours at 125°C. They observed that body weight gain, feed intake and feed conversion efficiency of chicks fed autoclaved rice bran were significantly higher than those chicks fed untreated rice bran. They concluded that autoclaving defatted rice bran hydrolyzed phytate phosphorus to inorganic phosphorus and improved phosphorus availability for chicks.

Said (1996) concluded that dry extrusion was found to be an effective means for processing waste material into high quality feed ingredients, not only resulting meals high in nutritive value but also these were microbiologically safe for inclusion into poultry diets. The extrusion cooking at 130°C of full fat or defatted rice bran improved its nutritive value. Ohtsubo and Yanase (1985) reported that as a result of extrusion cooking, the fine structure and gelatinization characteristics of defatted rice bran changed. The content of water soluble sugar increased.

Classen (1996) reported that enzyme hydrolysis activity was most effective in improving starch utilization however, presence of anti-nutritive
factors in cereals and digestive capacity of chick might affect the complex carbohydrate i.e. arabininoxylan utilization.

Carroll (1990) developed a dry heat extrusion method to stabilize rice bran. The process decreases moisture content and reduces the rate of rancidity development during storage. No chemicals or preservatives were used. Properties of the bran can be affected by rice variety, growing and storage conditions, milling and stabilization method. It has been successfully incorporated into bread, muffins, peanut butter and cookies up to 20%.

Fernando and Hewavitharana (1993) investigated effect of fluidized bed drying on the stabilization of rice bran. For this purpose rice bran was treated in a batch-fluidized bed at temperature from 90 to 130°C. It was observed that the fluidized bed treatment of rice bran requires much lower time for stabilization of rice bran compared with packed bed processes and therefore could be useful in commercial applications.

Young (2000) observed that heat treatment increased content of soluble dietary fiber and decreased insoluble dietary fiber in defatted rice bran. Wet heat increased water solubility index, water absorption index, water holding capacity, bulk density, hydration time and swelling of defatted rice bran and decreased its oil holding capacity. All heat treatments reduced bran lightness, increased redness and yellowness. Analysis showed that heat treated defatted rice bran had irregular and collapsed surfaces, indicated gelatinization of starch granules within the bran and modification of cell wall structure.

2.5 Physico-Chemical Characteristics of Wheat Flour

The whole wheat flour contained moisture 10.2-10.4%, protein 9.45-11.41% and ash 1.65-1.67% (Nagi et al., 1984). Similarly moisture 7.7%, protein 10.60% and ash 1.58% in whole-wheat flour was observed by Leelavathi et al. (1984). In an other study, it was found that ash content in
bread flour varied widely from 0.31-0.65%, protein content 9.6-11.2% and moisture content 10-13.6% that did not differ significantly amongst different flour samples (Parades et al., 1987). Taneja et al. (1983) analyzed four Indian wheat varieties and found that moisture, ash, crude protein, ether extract and crude fiber contents ranged between 7.88-8.96%, 1.43-1.79%, 10.11-11.77%, 1.74-2.93% and 0.98-1.42%, respectively. The nutritive value of wheat grains and whole wheat flour is almost identical. The factors like class, variety, environment i.e. climate, soil and cultural practices affect the composition of the wheat grain. The whole wheat flour generally contains moisture 13%, carbohydrate 69%, protein 12.2%, fat 2.3% and fiber 2.0% (FAO, 1989).

Ahmad (1993) analyzed whole wheat flour and reported moisture content 12.02%, crude protein 12.13%, crude fiber 1.92%, fat 2.30%, ash 1.65% and NFE 69.98%.

Ali (1996) analyzed patent wheat flour and found that it contained moisture 10.90%, crude protein 12.95%, crude fat 1.25% crude fiber 0.15%, ash 0.40% and NFE was 74.35%. Later on Ahmad (1997) tested whole wheat flour consists of dry matter 85.86%, protein 12.72%, fat 2.71%, ash 1.75% and crude fiber 2.62%.

Ayaz (1998) tested eight commercially available flour samples form different sources for their proximate composition and found that in these samples moisture, crude protein, crude fat, ash and NFE ranged from 10.25-13.31%, 9.23-12.81%, 1.25-1.49%, 0.31-0.64% and 72.13-78.23%, respectively.

Barkat (1998) tested commercial flour samples of seven flour mills for their composition. The protein content of the commercial flour ranged from 7.98-10.0%. While other components i.e. crude fat, moisture, ash and crude fiber ranged from 0.81-1.30%, 11.40-15.02%, 0.37-0.58% and 0.17-0.34%, respectively.
Haidery (2002) chemically analyzed commercial white flour and reported moisture content, crude protein, fiber content, crude fat, ash and NFE to be 11.20%, 9.77%, 0.37%, 1.13%, 0.52% and 76.96%, respectively.

Hoseney (1994) reported the chemical composition of patent wheat flour as protein 11.0%, ash 0.40% and fat 0.88%.

Iftikhar (2002) reported that wheat flour contains moisture 11.02%, ash 0.60%, protein 10.29%, fat 1.21%, fiber 0.16% and NFE 76.71%.

Pyler (1988) described the proximate composition of wheat flour as ash 0.5%, protein 11.0%, lipid 1.0% and crude fiber 0.5%.

Raymond (1993) described that soft flour contains protein 8.4-8.8%, ash 0.44-0.48%, fat 1.0% and NFE 76-77%. Hard flour contains protein 11.2-11.8%, ash 0.45-0.50%, fat 1.2% and NFE 74-75%.

Wheat flour stored improperly did not produce good quality chapati. The free fatty acids and alcoholic acidity increased in stored grains. The poor storage stability was due to higher moisture content (12.1%) as well as due to the activities of lipase, lipoxidase and protease. Adverse changes during storage were minimum when moisture level of flour was reduced from 12.1% to 7 or 8% (Leelavathi et al., 1984).

2.6 Supplementation of Rice Bran in Bakery Products

Baking is a developing industry in Pakistan. Now a days people are becoming more conscious about their health and nutrition. Foods that are convenient with good taste, reasonably priced and carry a favorable nutritional image are in great demand among bakery products especially cakes and cookies. Flour is the primary raw material, which provides a matrix into which other ingredients in varying proportions are mixed to form dough or batter in all soft wheat product formulations including cookies (Faridi, 1990). The functional and nutritional properties of rice bran have
appeared well suited to its usage as supplement in baked goods like bread, cookies and chapatis.

2.6.1 Bread

Bread is the most important commercial product of wheat. It is consumed as a staple food by most wheat eating people.

Rice bran replaced wheat flour at 15-30% in yeast bread and it was concluded that rice bran can be supplemented successfully up to 15% replacement level without affecting loaf weight, height or volume (Sharp and Kitchen, 1990). However, Hargrove (1990) discussed production, functional properties of rice bran and its applications in baked goods, pancakes, cereals, granola type bars, snacks and extruded foodstuffs.

Tangkanakal et al. (1995) made high fiber breads from wheat flour supplemented at 0, 5, 10, 15, 20, 25 and 30% levels with fiber obtained from nine different sources.

Holland et al. (1991) analyzed cereal grains and their products for proximate composition, nutritional and caloric assessment. Protein was 7.6% in white sliced bread while 8.5% in brown bread. Fiber contents were 1.5% in white bread as compared to 3.5% in brown bread. Values for Na, K, Ca and Mg were as follow, 530, 99, 100 and 20 mg/100g in case of white bread but for brown bread these values were 540, 170, 100 and 53 mg/100g, respectively.

Lima et al. (2002) studied the functional properties of bread made with processed full fat and defatted rice bran. Three cultivars (long, medium and short) were compared with control for 10 and 20% replacement of wheat flour, respectively. Loaf volume increased 2% for full fat and decreased 6% for defatted rice bran, 6% for full fat and 17% for defatted rice bran, respectively. Loaf volume was highest with medium rice bran and this was attributed to its lowest fiber content and highest starch content among three varieties. Texture profile analysis showed no
significant differences as far as cohesiveness and springiness but bread hardness, gumminess and chewiness increased with increased levels of rice bran and was higher for defatted rice bran bread than for full fat rice bran bread. Measurements of texture determined that there was no detrimental effect in adding 10% full fat to the bread and a very slight hardening of the loaves with 20% full fat rice bran when compared to the control.

Sharma and Chauhan (2002) concluded that addition of rice bran to wheat flour increased the contents of proteins, lysine and dietary fiber in bread and cookies proportionately to the level of supplementation. In addition to color, flavor, protein extractability, solubility of bran and other properties such as water and fat absorption, emulsifying and foaming capacity are important factors determining the potential use of bran in foods.

Taha et al. (1982) worked on enrichment of wheat bread with four protein sources, namely rice bran flour, fish protein concentrate, soy protein concentrate and sunflower seed protein concentrate. The protein sources were added at 5 and 10% levels. Chemical analysis of enriched bread revealed that protein content was increased in the range of 16 to 62% in different treatments. Mean values for sensory evaluation showed that protein sources were favorable supplements especially at 5% level.

Lynn (1969) concluded that defatted rice bran might be added at levels of 5, 10 and 15% in variety of especially baked products, yeast raised goods (pan bread and butter bread), quick breads and cookies after lipase inactivation. It also improved lysine content of bread and gave a bland flavor. Defatted rice bran increases dough yield, contributes to an attractive tan crumb and crust, does not disturb fermentation or mixing tolerance of dough, causes baked products to remain fresher, more moist and adds significant amino acids, minerals and vitamins to baked goods.
2.6.2 Cookies

A chemically leavened bread type product is known as biscuit. The term biscuit is used in the European countries and cookies in the United States of America. Biscuits and biscuit like products have been made and eaten by man for hundreds of years (Hoseney, 1994). Cookies are ideal for nutrient value, palatability, compactness and convenience. They differ from other baked products like bread and cakes because of having low moisture content which ensures that cookies are generally free from microbial spoilage and confer a long shelf life on the product (Wade, 1988). Nutritional and functional properties of defatted rice bran are well suited for baked products like cookies, muffins, breads, crackers, pastries, pancakes and waffles (Barber et al., 1981).

Kennedy et al. (1996) emphasized on new food products developed from rice bran. In this investigation, defatted rice bran and its oil were used to replace margarine and flour, respectively in bakery products. Sensory properties of these products were then determined. Banana muffins and peanut butter cookies were prepared using various proportions zero, low or high replacement levels of these fractions and sensory analysis was performed by a trained panel. Tenderness increased with rice bran meal level and decreased with rice bran fraction. It was concluded that rice bran fractions can be used to produce acceptable low fat, high fiber bakery products.

Zumbado et al. (1997) manufactured a traditional Cuban bakery product (Torticas de Moron), with 0 (control), 20, 25 or 30% of the usual white flour replaced by parboiled rice bran. Other ingredients included were butter, sugar, vanilla flavoring, salt and water. Protein, fat, crude fiber and ash were higher in the rice torticas than in the control. There were no significant changes in pH, acidity or net weight up to 72 hours of storage. It was further concluded that 25% replacement of flour with rice bran resulted
in a product with acceptable sensory properties, shelf life and chemical composition.

Sekhon et al. (1997) indicated that rice bran could be added to different food products to the extent of 5-10%. Stabilized full fat rice bran up to 20% level and unstabilized full fat or stabilized defatted rice bran up to 10% was found suitable in various food products (Singh et al., 1995).

Sharma and Chauhan (2002) supplemented wheat flour with rice bran stabilized by dry heat and extrusion cooking at levels of 5 to 20% in breads and cookies. Addition of rice bran to wheat flour increased the contents of proteins, lysine and dietary fiber in bread and cookies proportionately to the level of supplementation.

Semwal et al. (1996) studied the nutrients composition of biscuits commercially available in Mysore, India. In all biscuit types the protein contents ranged from 5.46 to 8.90%. Na, K, Fe and Ca varied from 800-4950, 450-1720, 38-230 and 120-1800 mg/kg, respectively. The Cu, Mn and Zn ranged from 1-7, 3.5-10.4 and 8.2-25.5 mg/kg, respectively. Total fat contents of the biscuits varied from 20.1 to 24.6% and total energy values ranged from 365 to 501 Kcal.

Ahmad (1996) reported that biscuits contained moisture, crude protein, crude fat, crude fiber, ash and NFE as 5.50%, 6.94%, 26.77%, 0.15%, 0.26% and 60.38%. Ahad (1999) reported moisture 1.42%, ash 0.48%, protein 7.47%, fat 21.64%, fiber 0.12% and NFE 68.87% in biscuits.

Akbar (2000) performed proximate analysis of cookies and found that moisture content of cookies was 3.99-4.06%, ash content 0.19-0.33%. crude fat 25.39-27.24%, crude protein 7.66-16.12%, crude fiber 0.20-0.28% and NFE 54.80-63.18%.
2.6.3 Chapati

In Pakistan, India and some other countries, flat bread called chapati or roti is major wheat based staple food product. Nan and leavened flat bread are also common, these are the least expensive and nutritionally most important products. Often, the terms chapati and roti are synonymously used to mean unleavened breads, produced from a dough prepared by mixing coarsely ground whole wheat flour and water. Though, it is not a source of nutritionally balanced protein, it is nonetheless a principal source of both protein and energy for the consumer. Chapati is normally consumed with a curry dish. In well-to-do families, the curry may consist of meat, vegetables and legumes, while in the very poor class often an onion or a piece of pickle with or without butter milk (lassi) may be sufficient. Since majority of the population in the Indo-Pakistan sub-continent belongs to the low income group therefore, their diet comprises primarily of chapati with less of the other nutritious food-stuffs.

In Pakistan about 90% of wheat is ground to make whole wheat meal flour locally called ‘atta’ with 100% extraction rate from which chapatis are prepared (Kent and Evers, 1994). Saxena and Haridas Rao (1995) found that tandoori roti made from whole wheat flour had softer texture and better flavor than commercial flour.

Chapatis are circular and unleavened flat breads which are staple food for inhabitants of Indian sub-continent and parts of the Middle East for centuries (Nurul Islam and Johansen, 1987). Chapatis are not only commonly eaten in Pakistan but also in India, Tibet, China and the Near East (Kent and Evers, 1994).

There are various forms of flat breads which are consumed around the world. The researchers have attempted to improve the product according to their own cultural requirements and eating habits. Chapati
dough consists of mainly flour and water containing small amounts of salt, fat or sodium bicarbonate (Faridi, 1988).

Wheat flour of 95-97% extraction rate is the most suitable for chapati making purposes (Chaudhry, 1968; Yasin et al., 1965). In general 80% extraction rate of flour or lower are preferred for the production of most types of flat breads. However the flours with extraction rate of 90% or higher are also used in certain locations especially in rural areas of the Middle East (Haq and Chaudhry, 1976).

Types of chapati include “Tandoori Roti” (bakes inside a mud oven), “Khameri roti” (containing yoghurt or buttermilk, sugar, salt and the dough allowed to ferment) while “Nan” made from white flour of 75% extraction rate by a yeasted sponge-and-dough process with the addition of sugar, salt, skimmed milk, ghee and gram flour or eggs (Chaudhry and Miller, 1970).

Generally chapati is prepared from whole-wheat flour obtained by grinding wheat in a disk mill (locally known as chakki). Conditions for preparation of chapati with respect to recipe, dough consistency, thickness, size, shape of the dough sheet and baking conditions vary widely in different regions and laboratories (Shurpalekar and Prabhavathi, 1976; Austin and Ram, 1971).

2.7 Biological Assay

Prakash and Ramanatham (1995) observed fractional classification of rice bran proteins, proximate composition and protein efficiency ratio (PER) of untreated and stabilized rice brans. Protein contents of defatted, milled and sieved bran flours ranged from 16.5-18.2%. Rice bran proteins were rich in albumins (32%) and globulins (26%). PER of acid stabilized rice bran was highest (2.18) followed by untreated (2.09), heat stabilized (2.03) and parboiled (1.99) rice bran. It was concluded that acid
stabilization of rice bran did not adversely influence protein quantity in food products.

Rice bran protein is relatively of high nutritional value, PER reported for rice bran generally ranged from 1.6 to 1.9 compared to casein value of 2.5. While for protein concentrates extracted from rice bran with dilute alkali, PER values ranged from 2.0 to 2.5. Digestibility of rice bran protein is 73% whereas for its concentrate is 90% (Saunders, 1990; Sayre et al., 1982). Later on, Dale (1997) also concluded that it is a good source of essential amino acids especially lysine and methionine.

Rice bran a by-product of rice milling industry is not being used directly as human foods, but mostly sold as poultry or animal feed ingredient and thus being consumed below its potential nutritive value. This is because the toxic or undesirable factors, which get accumulated in bran during polishing of rice and hinder the digestibility and availability of nutrients.

Marshall and Wadsworth (1994) reported that due to rice bran's overall composition, nutritional profile, functional characteristics and apparent hypoallergenicity, it has many applications in a diet, which is characterized by high dietary fiber and low saturated fat. It may be particularly beneficial to those individuals who are allergic to other cereal grains. However, strong evidences are available that the consumption of rice bran may be beneficial in reducing the risk of cardiovascular disease and colon cancer.

Majid (1997) fed diets containing defatted treated rice bran against the control ration. He gave acid, alkali, acid + alkali and extrusion treatments to rice bran. He concluded that out of four treatments extrusion cooked rice bran treated with 1% acetic acid showed significantly improved growth rate and feed utilization in birds as compared to other rations containing rice bran given other treatments.
Sayre et al. (1987) studied the nutritional qualities of raw and stabilized rice bran for broiler chicks and observed that the feed efficiency of full fat and stabilized rice bran was superior to defatted and stabilized rice bran.

Sayre et al. (1988) fed to meat strain chickens for 7-8 weeks the diets containing rice bran 600g/Kg. They observed that extrusion cooking of rice bran (10 min at 130°C) stabilized it and improved weight gain and feed efficiency for 1st two weeks of feeding but advantage was lost by the end of feeding period. Addition of calcium 10g/Kg to stabilized rice bran prevented decrease in performance after 2 weeks and chickens fed this diet continued to gain weight at an increasing rate till end of experiment.

Deolankar and Singh (1979) fed diets to the chicken for 10-49 days of age based on maize and groundnut oil meal without or with untreated, autoclaved or dry heated rice bran replacing 50, 75 or 100 percent maize. All diets contained 22 percent protein. They observed significantly lower body weights with 100 percent autoclaved rice bran than with all other treatments and significantly higher without rice bran with 50 percent untreated and dry heated rice bran than with all other treatments. Feed conversion efficiency was significantly higher with 50 percent dry heated rice bran than with all other treatments except with 75 percent autoclaved, 75 and 100 percent untreated rice bran. Feed efficiency of the 100 percent autoclaved rice bran group was significantly lower than that with all other treatments. They noted that weight of pancreas and liver was not significantly affected by any of the brans.

Malik (1976) studied the effect of feeding different levels of rice polishing (solvent extracted) on the growth rate and feed utilization of broiler chicks using 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 30.0, 40.0 and 50.0 percent level without affecting the protein content. He reported that 10 percent level of rice bran could be used in broiler ration without impairing
the growth rate and feed utilization. He also noticed no marked effect on the weight of internal organs.

Gupta and Singh (1988) fed broiler chickens for 5 weeks diets based on maize, untreated, autoclaved and deoiled rice bran without or with 8 percent rice bran oil or a protein supplement. They reported that feeding deoiled rice bran reduced growth, which was not improved by autoclaving or adding oil but was partly improved by increasing dietary protein to 26 percent. They further reported that feed intake was higher and feed efficiency poorer in chickens given rice bran compared with maize.

Eshwaraiyah et al. (1986) in 2 experiments fed caged broilers on maize based diet without or with 16, 32 and 48 percent maize replaced by raw rice bran (RRB), deoiled rice bran (DRB) or parboiled rice bran (PRB). In experiment 1, all diets were equal in nitrogen value and in experiment 2, equal in nitrogen and energy value. They observed that in 1st experiment average body weight gain to 4 weeks old with RRB as 16, 32 and 48 percent was 562, 552 and 450g; with DRB 583, 582 and 480kg; with PRB 547, 517 and 486g. With basal diet the gain was 549g. Feed intake with RRB was 1.84, 1.93 and 2.42; with DRB 568, 591 and 542 and with PRB was 595, 576 and 479g; with control diet gain was 574g. Feed intake with RRB was 1.80, 1.83 and 1.91; with DRB 1.74, 1.74 and 1.90; with PRB 1.67, 1.83 and 1.86 and with control was 1.71-g/g gain. They further reported that RRB and DRB seemed to induce pancreatic hypertrophy.

Tiemoko (1992) conducted experiments on broiler chickens for 6 weeks and fed iso-nitrogenous, iso-energetic diets formulating with 0, 15 and 30% rice bran replacing maize, wheat shorts and soybean meal. He observed significant improvement in live weight gain but feed conversion and efficiency was unaffected. Feed intake tended to increase with inclusion of rice bran in diets but differences between treatments were not
significant. The use of rice bran in broiler diets reduced feed cost per Kg weight gain.

Chauhan and Sharma (1996) fed broiler chicks diets based on maize, which were replaced by rice bran at 0(T₁), 25(T₂), 50(T₃), 75(T₄) and 100% (T₅). They observed that body weight and feed conversion efficiency differed significantly (P<0.05) among the treatments. T3 diets resulted in higher (P<0.05) body weight compared with control diet. Feed intake increased with rice bran level up to 50%. Feed conversion efficiency was similar in T₁, T₂ and T₃ diets. Cost of production per live weight was lowered in T₃ diet. It was concluded that replacement of maize with rice bran up to 50% level does not affect the growth of chicks.

Young and Jae (2001) investigated effects of 2 dietary fiber sources, rice bran and wheat bran on tissue lipid levels and large bowel fermentation in male Sprague Dawley rats. Feed efficiency ratio and body weight gain were not significantly different between the experimental groups. Non-fasted plasma total cholesterol was elevated in rats fed rice bran. Rice bran and pectin had the same effect in increasing the total lipid content of faeces but rice bran was more effective in the faecal excretion of bile acids than pectin or wheat bran. Results indicated that the source of dietary fiber was closely related to short chain fatty acid production and intestinal physiology.
Chapter-III

MATERIALS AND METHODS

3.1 Raw Materials

Rice bran of Basmati-385 was collected from Reem Rice Mills (Pvt.) Limited, Muridke, Sheikhupura, Pakistan soon after the milling. The remaining ingredients for the preparation of bread, cookies and chapatties etc. were purchased from the local market.

3.2 Processing of Rice Bran

After milling, the processing of rice bran was carried out immediately to inactivate endogenous lipases, hydrolyze phytic acid into myo-inositol and available phosphorus, denature the trypsin inhibitor and haemaglutin-in-lectin proteins to inactive their anti-nutritional activities without damaging the protein quality of rice bran. To achieve all these objectives, the rice bran was subjected to various chemical and physical treatments.

3.2.1 Chemical Treatment

Different chemical treatments were given to the rice bran are described below:

RRB: Rice bran termed as raw rice bran was left untreated and used as control.

PRB-I: In the first treatment, rice bran was thoroughly soaked with simple water in a mechanical mixer for 15 minutes to adjust its moisture content up to 20%.

PRB-II: In the second treatment, rice bran was homogeneously mixed with 20% (w/w) solution of 1% acetic acid.

PRB-III: In the third treatment, rice bran was uniformly mixed with 20% (w/w) solution of 1% calcium hydroxide.
3.2.2 Physical Treatment

Except RRB, all the treated samples of rice bran (PRB-I, PRB-II and PRB-III) were passed through an extruder cooker maintained at a temperature of 130±2°C for 10-15 sec. All the samples were dried to a moisture level less than 10% by using Hot air oven.

3.2.3 Packaging and storage

The rice bran was packed in air impermeable plastic bags with free oxygen absorber (FOA) following the procedure of Tanaka et al., (2000) and stored at room temperature. These samples were further used for chemical assay, preparation of various food, feed and their biological evaluation.

3.3 Lipase Activity

The activity of endogenous enzyme lipase was determined according to the method of AOCS, 1998 by estimating the amount of free fatty acids (FFA) in rice bran. Increase in FFA was taken as function of lipase.

3.4 Peroxidase Activity

Peroxidase activity of raw and processed rice bran was assayed by the method of Vetter et al. (1958). Residual peroxidase activity in processed rice bran was calculated on dry weight basis as percentage of activity in raw bran (100% activity) sampled during the same processing run. Peroxidase inactivation was used to give a rapid indication of the effects of processing, as it is considered to be one of the more heat stable plant enzymes (Barber et al., 1974) and lipase inactivation was assumed to precede if not parallel that of peroxidase.

3.5 Trypsin Inhibitor Activity

One gram of sample was blended with 15 ml of 0.05N HCl in a Sorvall Omni Mixer (Ivan Sorvall, Inc., Newtown). The extracted slurry was then centrifuged and trichloroacetic acid (TCA) was added
to the supernatant and again centrifuged. After neutralization the enzyme inhibitory activities were determined as described by Decker (1977).

3.6 Haemagglutinin-lectin Activity

Haemagglutinin activity of raw and processed rice bran samples was determined by rabbit erythrocyte agglutination test (Benedito and Barber, 1978). Lectin is measured in haemagglutinin units (HU). Lectin activity of raw and processed rice bran was assayed as reported by Tan et al. (1983).

3.7 Phytates

Phytic acid content of raw and processed rice bran samples was determined by following the method of Haug and Lantzesch (1983). To determine phytate, extracted sample was heated with acidic ammonium iron-III sulphate solution of known content. The decrease in iron content (determined colorimetry with 2, 2 bipyridine at 519 nm) in supernatant was the measure of phytate content. The same procedure was also followed for standard phytate solution for each set of analysis.

3.7.1 Phosphorus Estimation

Phosphorus was estimated in different rice brans by ashing a weighed amount of sample at 550°C. The phosphorus was estimated through colorimetry by developing color with ammoniated molybdate solution according to the method of AOAC (2000).

3.7.1.1 Total Phosphorous

Total phosphorous was estimated in different rice brans by ashing a weighed sample at 550°C and dissolving the ash in 6 N HCl, heating, boiling and filtration. The volume was made up with distilled water. 10 ml of the solution was taken, neutralized with NH₄OH. The phosphorous was
estimated through colorimetry by developing color with ammoniated molybdate solution according to the method of AOAC (2000).

3.7.1.2 Available Phosphorous

Available phosphorous was determined by extracting a weighed portion of raw and processed rice bran in neutral ammonium citrate according to the method of Day et al. (1973).

3.8. Microbial Load

3.8.1 Mould Count

Mould counting in raw and processed rice bran was made by serial dilution on agar plate technique on sabouraud agar medium (Beneke, 1962).

3.8.1.1 Preparation of media (Sabouraud and agar)

The basic ingredients of media were dextrose 40 g, peptone 10 g and agar 35 g. These ingredients were mixed in 1000 ml distilled water to prepare the media.

3.8.1.2 Sterilization

Sterilization of the media was done in autoclave at 121°C under 15 lb pressure for 15 minutes and stored in a refrigerator.

3.8.1.3 Sampling

One-gram sample of each treatment was taken aseptically and put in dilution bottle and made volume 100 ml by distilled water to prepare 1:100 dilutions. 1 ml of this dilution was poured in the triplicate disposable petri dishes from each sample. 15 ml of molten media was also poured in each. Dilution and media were mixed by swirling the petri dishes to and forth, allowed to solidify and petri dishes were inverted to avoid condensation of moisture inside the cover.
3.8.1.4 Incubation

Petri dishes carrying samples were incubated at 30°C for 72 hours. After 72 hours of incubation period, colonies developed in petri dishes were counted.

3.8.2 Total Viable Count

Nutrient agar medium (Lowry and Gill, 1994) consisting of peptone 6.0, casein hydrolyzate 4.0, yeast extract 3.0, glucose 2.0, beef extract 1.5 and agar 15.0 g/l was used for the determination of bacterial count in raw and processed rice brans.

3.9 Neutral Detergent Fiber (NDF)

One gram of sample was taken in a conical flask and 100 ml of neutral detergent solution (18.6 g EDTA and 8.6 g sodium tetra borate) were mixed in 100 ml distilled water to form the solution-1. Then 30 ml sodium lauryl sulphate and 10 ml ethoxy ethanol were added in it. Solution-2 was prepared by adding 450 g of di-sodium hydrogen phosphate in 100 ml water. Both the solutions were mixed to form one liter NDF reagent and 0.5 g sodium sulphite were added to it. The contents were boiled for one hour, cooled and filtered. The residue was washed 5-6 times with hot water at 80°C and then with acetone. Then the residue was oven dried at 105°C for three hours and weighed after cooling. The NDF percentage was calculated as given below by follow the method of Van and Robertson (1985).

\[
\% \text{ NDF} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100
\]

3.10 Acid Detergent Fiber (ADF)

One gram of sample was taken in a conical flask and 100 ml ADF reagent (20 g of acetyl trimethyl ammonium bromide was dissolved in sufficient 1N H₂SO₄ to make 1 liter ADF reagent) was added to it. The contents were boiled for one hour than cooled and filtered. The residue was washed with hot distilled water and then with acetone. Then the
residue was placed in china dish and dried at 105°C for 3 hours in an oven. Weight of the residue was recorded and ADF percentage was worked out with the following formula as detailed by Van and Robertson (1985).

\[
\% \text{ ADF} = \frac{\text{Weight of the residue}}{\text{Weight of the sample}} \times 100
\]

3.11 Bulk Density

The bulk density of different rice bran samples was determined according to the method of Egan et al. (1981) by filling the samples in a cylinder, after shaking and vibrating the volume of 50 ml was completed and then weighed. Bulk density was calculated by weight per unit volume.

3.12 Chemical Evaluation

All the rice bran samples were analyzed for moisture content, crude protein, crude fat, crude fiber, ash and NFE according to their respective methods as described in AACC (2000).

3.12.1 Moisture content

The moisture content of each sample was determined by drying 3 g sample in an air forced draft oven at a temperature of 105±5°C till to a constant weight (AACC, 2000).

3.12.2 Crude Protein (CP)

The crude protein content was determined in each sample by kjeldahl's method as described in AACC (2000). The protein percentage was calculated by multiplying nitrogen with respective conversion factor.

3.12.3 Crude Fat

The crude fat or ether extract (EE) was determined by using Soxhlet System HT-2, Extraction Unit, Tecator, Hoganas, Sweden according to the procedure given in AACC (2000).
3.12.4 Crude Fiber (CF)

The crude fiber content was estimated by digesting the fat free samples in 1.25% $\text{H}_2\text{SO}_4$ followed by 1.25% NaOH solution as mentioned in AACC (2000).

3.12.5 Ash

The ash content in different samples was estimated by incinerating the dry samples in a muffle furnace at a temperature of 550°C by following the procedure of AACC (2000).

3.12.6 Nitrogen free extract

Nitrogen free extract was calculated according to the following expression.

\[ \text{NFE} = 100 - (\text{Moisture} + \text{CP}\% + \text{EE}\% + \text{CF}\% + \text{Ash}\%) \]

3.13 Starch

After sample preparation starch in various rice bran samples was determined by di-nitro silicic (DNS) acid through spectrophotometer at 550nm absorbance according to procedure of AOAC (2000).

3.14 Bakery Products

Different bakery products i.e. bread, cookies and chapaties were prepared by the addition of processed rice bran having minimum anti-nutritional factors in different combinations with wheat flour as mentioned in Table 1.
Table 1. Treatments for the preparation of bakery products from wheat flour and rice bran

<table>
<thead>
<tr>
<th>Wheat flour* (g)</th>
<th>Processed Rice bran (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

* Straight grade flour for the preparation of bread and cookies while whole wheat flour for chapattis was used.

3.14.1 Preparation of Bread

The bread was prepared by straight dough method from the processed rice bran supplemented flour samples (Table 1) according to the method of AACC (2000). The bread was evaluated for external and internal characteristics subjectively by a panel of 5 trained judges following the instructions of Larmond (1977) with some modifications.

3.14.2 Preparation of Cookies

The cookies were prepared from the best processed rice bran supplemented flour according to the method of AACC (2000). After creaming and thorough mixing, homogeneous mass was sheeted to uniform thickness. Cookies cutter was used for cutting the rolled dough sheet. The cookies were baked at a temperature of 220°C for about 10 minutes. Sensory evaluation of cookies was also carried out for color, crispness, taste, texture and total scores by following the procedure of Pyler (1988).
3.14.3 Preparation of Chapaties

The whole wheat flour was mixed with processed rice brans at 0, 5, 10, 15, 20, 25 and 30% levels. The chapaties were prepared from samples according to the procedure described by Haridas Rao et al. (1986). Its dough was prepared by mixing 200 g of flour in predetermined amount of water for 3 minutes in a mixer (National Mfg.Co., Lincoln, Nebraska) and allowed to rest for an hour at room temperature.

A portion of dough weighting 80 g was rounded and turned into chapati by using specially designed platform to facilitate the rolling of dough. The chapaties were of uniform thickness and baked on a thermostatically controlled hot plate at a temperature of 210°C for 2 minutes. Sensory evaluation of chapaties was performed for color, flavor, taste, texture, foldability, breakability and total scores according to the method of Meilgaard et al. (1991).

3.15 Selection of the Best Treatment

Based on the results of sensory evaluation of bakery products, three best treatments along with control were selected by the panel of judges. These samples were further used for nutritional assay studies.

3.16 Biological Evaluation of Chapaties

The biological evaluation of rice bran supplemented chapaties was done by feeding it to young albino rats. The composition of diets is shown in Table 2. Each diet contained 10% protein.
### Table 2. Composition of various diets containing chapaties

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Casein</th>
<th>C₀ 100% wheat flour</th>
<th>C₁ 95+5</th>
<th>C₂ 90+10</th>
<th>C₃ 85+15</th>
<th>Non protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>10.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PRB supplemented chapaties</td>
<td>-</td>
<td>72.5</td>
<td>72.0</td>
<td>70.5</td>
<td>69.0</td>
<td>-</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>7.5</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral premix*</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>67.3</td>
<td>10.0</td>
<td>10.5</td>
<td>12.0</td>
<td>13.5</td>
<td>80.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

* The composition of Vitamin and Mineral Mixture is given in Appendices I & II
The feeding trials were conducted according to the procedure of Thomas Mitchell balance methods and modified by Eggum (1976). The colony of rats was maintained at Pakistan Council of Scientific and Industrial Research (PCSIR) biological experimental station, Lahore. Twenty-four weanling albino rats of 3 weeks age were used for the nutritional evaluation of the experimental diets. The rats were fed on basal diet for a period of 1 week and then randomly divided into 6 groups comprising of 4 rats each. All the experimental diets (Table 2) were randomly allotted to each group. The diets were prepared in such a way that 10% protein was available from each diet. A separate filter paper sheet was used for each group in a tray for the collection of excreta. Fresh and clean water was provided all the times to each group in separate glass bottles. All the groups were fed ad-libitum for a period of 10 days. The following nutritional parameters were derived as given below:

3.16.1 Feed Consumption and Body Weight

Feed consumption of different groups of rats was measured on daily basis. The weight of each group was also recorded on daily basis. The record of feed intake and body weight was maintained.

3.16.2 Protein Efficiency Ratio (PER)

PER of different experimental diets was determined from weight gain and protein intake data of rats as described below:

\[
\text{PER} = \frac{\text{Gain in weight}}{\text{Protein intake}}
\]

3.16.3 True Digestibility (TD)

After 2 days feeding trials, the faeces were collected from each cage then dry weight and nitrogen content were determined. The
nitrogen intake was calculated from the feed consumption and then TD was determined as given below:

\[
\text{Nitrogen intake} - (\text{Faecal N-Metabolic N})
\]
\[
\text{TD} = \frac{\text{B-Bk + Ik}}{1} \times 100
\]
\[
\text{Nitrogen intake}
\]

3.16.4 Net Protein Utilization (NPU)

The nitrogen content of diets, faeces and carcass of each group including protein free group in duplicate were determined by micro kjeldahl’s method. The net protein utilization was determined by the method of Miller and Bender (1955) as shown below:

\[
\text{NPU}\% = \frac{\text{B-Bk + Ik}}{1} \times 100
\]

Where
\[
\begin{align*}
\text{B} &= \text{Body N of test group} \\
\text{Bk} &= \text{Body N of protein free group} \\
1 &= \text{N intake of test group} \\
\text{Ik} &= \text{N intake of protein free group}
\end{align*}
\]

3.16.5 Biological Value

It was calculated by applying the following formula:

\[
\text{Net protein utilization} \\
\text{BV}\% = \frac{\text{Net protein utilization}}{1} \times 100 \\
\text{True digestibility}
\]

3.17 Experiments on Broiler Chicks for Nutritional Evaluation

The nutritional evaluation of rations containing raw and processed rice bran (Table 3 & 4) was carried out by using one day old Hubbard broiler chicks. Two experiments were conducted using 150 Hubbard one day old broiler chicks in each experiment. The data on weight gain, feed consumption and feed conversion ratio (FCR) etc. were collected during the experimental period of 8 weeks. The
data were subjected to statistical analysis according to the completely randomized design (one way analysis of variance). The random observations thus obtained were assumed to follow the statistical model given below:

$$X_{ij} = \mu + D_i + e_{ij}$$

Where

- $i = 1, 2, 3, \ldots \ldots$ (number of rations)
- $j = 1, 2, 3 \ldots \ldots$ (number of observations on each ration)
- $X_{ij}$ = jth observation on ith treatment
- $\mu$ = Population mean
- $D_i$ = Effect of ith ration
- $e_{ij}$ = The random error associated with jth observation on ith treatment

### 3.18 Statistical Analysis

The data obtained from various parameters of processed rice bran supplemented various products i.e. bread, cookies and chapatis etc. and for the nutritional trials were subjected to statistically analysis using one way analysis of variance technique according to the method of Steel et al. (1997).
Table 3. Composition of experimental rations for maize supplementation with rice bran

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A Control</th>
<th>B RRB As such</th>
<th>C PRB-I Extruded</th>
<th>D PRB-II Acid</th>
<th>E PRB-III Alkali</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>31.5</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Processed Bran Rice</td>
<td>-</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Wheat</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Broken Rice</td>
<td>17</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cotton Seed Meal</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Soybean Meal 44%</td>
<td>14</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Maize Gluten Meal 60%</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Wheat Bran</td>
<td>1.75</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Beef Tallow</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Fish Meal 55%</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cane Molasses</td>
<td>1.91</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amino Acid Lysine++</td>
<td>0.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin &amp; Mineral Premix</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4. Composition of experimental rations for wheat supplementation with rice bran

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A Control</th>
<th>B RRB As such</th>
<th>C PRB-I Extruded</th>
<th>D PRB-II Acid</th>
<th>E PRB-III Alkali</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Wheat</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Broken Rice</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Processed Rice Bran</td>
<td>-</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Maize Gluten Meal 60%</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cotton Seed Meal</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Soybean Meal 44%</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Guar Meal</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Poultry By-Product</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Fish Meal 55%</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cane Molasses</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Bone Meal</td>
<td>0.85</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amino Acid Lysine++</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin and Mineral Premix</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
4.1 Proximate Composition of Processed Rice Bran

The effect of processing was studied on the chemical composition of rice bran. The results of statistical analysis have been presented in Table 5. The data for proximate composition i.e. moisture, crude protein, crude fat, crude fiber, ash and NFE have been shown in Table 6.

The mean squares for proximate composition of processed rice bran revealed statistically significant ($P \leq 0.01$) differences among moisture content of different rice brans while crude protein, crude fat, crude fiber, ash and NFE showed non-significant differences. The results indicated that processing of rice bran showed no significant effect on crude protein, crude fat, crude fiber, ash and NFE contents of various rice brans (Table 5).

The differences among moisture content of processed rice brans ($T_2$, $T_3$ and $T_4$) were statistically ($P \leq 0.05$) non-significant while $T_1$ (raw) showed significantly higher moisture content. The variation in moisture content was due to careful drying of processed rice brans (PRB) below 10% moisture level (9.30 to 9.75%) while unprocessed rice bran contained more moisture due to non drying.

The results pertaining to proximate composition of processed rice bran were in conformity with the findings of Farrell (1994) who found protein 11–17%, fat 11–18%, fiber 10%, ash 9% and nitrogen free extract 45–65% in rice bran. Similarly Pomeranz and Oryl (1982) reported that rice bran had protein 13.2–17.3%, crude fiber 9.5–3.2% and ash 9.2–11.2% on dry weight basis. Like wise Warren and Farrell (1990) reported that the crude protein ranged from 13.4–17.4%, ether extract 20.4–23.4% and ash
Table 5. Mean squares for proximate composition of processed rice brans

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Moisture</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>3.292**</td>
<td>0.092&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.237&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.152&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.190&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.305&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.249</td>
<td>0.432</td>
<td>0.438</td>
<td>0.140</td>
<td>0.278</td>
<td>6.364</td>
</tr>
</tbody>
</table>

** = P≤0.01
NS = Non significant
Table 6. Means for proximate composition of processed rice brans

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Crude Protein (%)</th>
<th>Crude Fat (%)</th>
<th>Crude Fiber (%)</th>
<th>Ash (%)</th>
<th>NFE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>11.50a</td>
<td>13.00a</td>
<td>12.93a</td>
<td>7.65a</td>
<td>10.34a</td>
<td>56.08a</td>
</tr>
<tr>
<td>T₂</td>
<td>9.50b</td>
<td>13.40a</td>
<td>13.60a</td>
<td>7.71a</td>
<td>10.58a</td>
<td>54.71a</td>
</tr>
<tr>
<td>T₃</td>
<td>9.75b</td>
<td>13.25a</td>
<td>13.35a</td>
<td>7.25a</td>
<td>10.50a</td>
<td>55.69a</td>
</tr>
<tr>
<td>T₄</td>
<td>9.30b</td>
<td>13.28a</td>
<td>13.30a</td>
<td>7.55a</td>
<td>10.95a</td>
<td>54.92a</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = RRB (Raw)
T₂ = PRB-I (20% moist and extruded)
T₃ = PRB-II (20% moist with 1% sol. of acetic acid and extruded)
T₄ = PRB-III (20% moist with 1% sol. of calcium hydroxide and extruded)
10.5% among several cultivars of rice bran grown in Australia. The present study is also in concordance with the findings of Saunders (1990) who illustrated that stabilized and parboiled rice bran had moisture 12%, protein 13%, fat 16%, crude fiber 9% and ash 10%. Later on Al–Jassar and Al–Mustafa (1996) showed normal level of protein (12.56%) and fat (15.15%) in rice bran.

The present results are also identical to the results reported by Malik and Chughtai (1979) and Gohl (1981) except crude fiber which was higher in the present investigation and it might be due to varietal and processing conditions (Rao and Reddy, 1986).

4.2 Chemical Composition of Processed Rice Bran

The data regarding chemical composition such as starch, total phosphorus, available phosphorus, increase in phosphorus availability, acid detergent fiber, neutral detergent fiber and bulk density have been presented in Table 8a. The analysis of variance results in Table 7 showed statistically non-significant differences for starch content and total phosphorus among different rice brans. With respect to available phosphorus, increase in phosphorus availability, acid detergent fiber, neutral detergent fiber and bulk density, significant differences existed among different rice brans.

The results indicated that available phosphorus ranged from 0.34 to 1.64% in different rice brans. All the treatments differed significantly with one another. The processing increased the phosphorus availability by 50.27, 89.13 and 76.40% for treatments T2, T3 and T4, respectively. Acid treated rice bran yielded significantly the highest increase in available phosphorus and the lowest was in raw rice bran. The processing of rice bran with various treatments decreased the acid detergent fiber (ADF). The ADF in T1 (raw rice bran) was found to be significantly higher than
<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Treatments</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td></td>
<td>0.470&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.605</td>
</tr>
<tr>
<td>Available Phosphorus</td>
<td></td>
<td>0.001&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.007</td>
</tr>
<tr>
<td>Increase in Phosphorus</td>
<td></td>
<td>2936.203&lt;sup&gt;**&lt;/sup&gt;</td>
<td>10.418</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td></td>
<td>56.840&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.501</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td></td>
<td>3.052&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.241</td>
</tr>
<tr>
<td>Bulk density</td>
<td></td>
<td>0.198&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
</tbody>
</table>

NS = Non significant
** = P<0.01
Table 8a. Means for chemical composition of processed rice brans

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Starch (%)</th>
<th>Total Phosphorus (%)</th>
<th>Available Phosphorus (%)</th>
<th>Increase in Phosphorus availability (%)</th>
<th>Neutral detergent fiber (%)</th>
<th>Acid detergent fiber (%)</th>
<th>Bulk density (g/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>15.25a</td>
<td>1.80a</td>
<td>0.34d</td>
<td>18.89d</td>
<td>30.23a</td>
<td>11.21a</td>
<td>0.39c</td>
</tr>
<tr>
<td>T₂</td>
<td>16.00a</td>
<td>1.85a</td>
<td>0.93c</td>
<td>50.27c</td>
<td>24.62b</td>
<td>9.87b</td>
<td>0.82b</td>
</tr>
<tr>
<td>T₃</td>
<td>15.50a</td>
<td>1.84a</td>
<td>1.64a</td>
<td>89.13a</td>
<td>20.52c</td>
<td>8.91b</td>
<td>0.85b</td>
</tr>
<tr>
<td>T₄</td>
<td>15.05a</td>
<td>1.78a</td>
<td>1.36b</td>
<td>76.40b</td>
<td>21.63c</td>
<td>9.23b</td>
<td>0.91a</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁  =  RRB (Raw)
T₂  =  PRB-I (20% moist and extruded)
T₃  =  PRB-II (20% moist with 1% sol. of acetic acid and extruded)
T₄  =  PRB-III (20% moist with 1% sol. of calcium hydroxide and extruded)
processed rice brans. However, statistically non-significant differences among processed rice brans were observed.

The neutral detergent fiber (NDF) of raw rice bran was 30.23%, which was significantly higher than all other processed rice brans by 18.56, 31.99 and 28.45%, respectively (T₂ to T₄). The minimum NDF was found in T₃ (20.52%) so, the processing reduced the NDF contents from 30.23 to 20.52%. The comparison of means showed that T₃ and T₄ were statistically non-significant.

The processing of rice bran showed an increase in bulk density from 0.39 to 0.91 g/cc. The increase was by 110.3, 118.0 and 133.3% for T₂, T₃ and T₄, respectively. Comparison of means showed that raw rice bran (T₁) differed significantly from all other processed rice bran treatments. The T₂ and T₃ treatments of processed rice bran showed non-significant difference but these exhibited statistically significant differences from rest of the treatments (T₁ and T₄).

The results of the present study favor the conclusions drawn by Saunders (1990) who reported that rice bran contains 10–20% starch which get included due to breakage of endosperm during milling but its quantity varied due to amount of breakage and degree of milling. These findings were also substantiated by Farinu (1989) who reported that soft rice bran contains 18% soluble carbohydrate but Marshall and Wadsworth (1994) further supported the present study. Their results revealed that rice bran contained 15.8% starch in the form of amylose and amylopectin. For the present investigation, the rice bran was moistened with 20% water, the starch granules also absorbed water and on extrusion cooking, it was found to be gelatinized. By this process, rice bran was coated with a thin starchy film by giving PRB a crispy structure and protective layer. This encapsulated starch layer can prevent the rice bran from moisture and atmospheric oxygen. The starch modification by extrusion assured the
availability of proteins and B-complex vitamins, particularly thiamine as 78% of the original thiamine was present in rice bran (Saunders, 1990). Each treatment (20% moist and extruded, acetic acid or calcium hydroxide plus extruded) gave a specific structure to PRB. According to Sharp (1981), Tollefson and Bice (1972), the processed rice bran indicated less gelatinization. The crumbs produced by PRB (acid or alkali) were less firm as compared to raw rice bran.

Increase in phosphorous availability in raw rice bran was very low (18.89%). On processing its availability increased by 50.27, 89.13 and 76.40% for T₂, T₃ and T₄ treatments, respectively. The acetic acid treatment ranked to the top (89.13%). This revealed that on acidic pH, phytate phosphorous was hydrolyzed more than alkaline (calcium hydroxide) and neutral (plain water). Like wise other complex minerals, proteins would had been released as reported by Luh et al. (1991), converting the phytates into inositol molecules, free minerals and protein etc. According to Saunders (1990), rice bran is rich in minerals but its range depends on rice variety, degree of milling and growing environment.

The present investigation showed that NDF and ADF decreased, indicating that more nutrients were available after processing of rice bran by various treatments. The results coincide with the study presented by Warren and Farrell (1990) who showed that neutral detergent fiber (25.6%) and acid detergent fiber (12.2%) were present in several cultivars of rice bran grown in Australia. The results also appear to agree with Saunders (1990) who reported that total dietary fibers were in the range of 20–25% in stabilized full fat rice bran. Fiber is also considered as anti-nutritive factor because of its ability to bind mineral cations (Rasper, 1979). The non fermentable carboxymethyl cellulose reduces digestion of fat, protein and starch. Similarly Purosothaman et al. (1989) revealed reduction in hemicellulose and cellulose by 47.9 and 36% and increased available
carbohydrates above 30% by 0.34N HCl followed by heat treatment of
decooled rice bran. In another study, Purusothaman et al. (1990) indicated
the effects of autoclaving on the defatted rice bran (120°C for 3 min.)
decreased cell wall constituents i.e. hemicellulose 43.9%, cellulose 9.9%
and increased total cell contents by 28.1%.

4.3 Storage of Rice Bran

The processing of rice bran was accomplished to protect the rice
bran from enzymatic degradation, microbial spoilage and insect attack.
Adequate storage is necessary to protect it from recontamination and
reversion of lipase or peroxidase activity. However, oxidative rancidity
might be a reason for the deterioration of the stored samples that could be
controlled by proper packaging and controlled storage condition. The
storage of PRB was done in air impermeable polypropylene bags with free
oxygen absorber (FOA) for further use.

4.4 Anti-nutritional Factors of Rice Bran

4.4.1 Lipases

Lipases of raw and processed rice bran samples (moist extruded,
acetic acid or Ca(OH)₂ treated and extruded) were analyzed for free fatty
acid (FFA). The FFA formation is a criteria to determine the lipase activity
and evaluation stability. Free fatty acids for raw and processed rice brans
have been presented in Fig 1.

It is obvious that FFA in PRB, PRB-I, PRB-II, and PRB-III varied
from 0.08 to 3.20, 0.07 to 0.20, 0.01 to 0.08 and 0.04 to 0.15%,
respectively during 90 days storage that showed an increasing trend with
the passage of time till the end of the study period. The highest value for
FFA was recorded for RRB while the minimum amount for this parameter
was observed in PRB. Among the processed rice bran samples least value
for FFA was observed in acetic acid treated rice bran (PRB-II) than that of
calcium hydroxide treated rice bran (PRB-III). The FFA of processed rice
Fig. 1 Free Fatty acids of Rice Brans

- T1=PRB(Raw)
- T2=PRB-I(20% moist and extruded)
- T3=PRB-II(20% moist with 1% solution of acetic acid and extruded)
- T4=PRB-III(20% moist with 1% solution of calcium hydroxide and extruded)
bran (dry) was higher than PRB (moist) because such conditions facilitate the enzymatic activity.

The results of existing study were corroborated with the previous work of Barber and Benedito-de (1980), Sayre et al. (1982) and Randall et al. (1985), they concluded from their research that extrusion for a short time was much effective for enzyme inactivation. The PRB needed to be dried below 10% moisture content for safe storage to get rid from the microbial activities. As the fat in paddy are quite stable but milling operation enhances the lipases and lipo-oxygenases activity, moreover unsaturated fats are prone to oxidation that leads to the formation of undesirable fatty acid as reported by Desikacher, 1974, Ryu and Cheigh, 1980 and Farrell, 1994.

4.4.2 Peroxidase

The results of peroxidase have been presented as absorbance unit per gram of PRB and compared with raw rice bran (RRB) that is considered as 100. The peroxidase was not completely and irreversibly inactivated or denatured by high temperature for short time (HTST) treatment in contrary to the microbes as it had a direct relationship with off flavor in foods. It is evident from the Fig. 2 that the peroxidase activity decreased in all the processed rice brans at 0, 5, 10 and 15 seconds interval, respectively. The diminishing trend from 47 to 12, 40 to 2 and 43 to 8 in PRB-I, PRB-II, and PRB-III, respectively during 15 seconds was recorded. Acetic acid treated rice bran (PRB-II) showed maximum decrease than that of calcium hydroxide treated rice bran (PRB-III).

The results pertaining to present investigation are in concord with the work of other researchers like Randall et al. (1985) and Sayre et al. (1982) who accomplished inactivation of peroxidase by dry heat and by adding up of varying amount moisture. Furthermore, Barber et al. (1978) also found that if dry heat is applied, the peroxidases become active again
Fig. 2 Effect of Processing on Peroxidase activity of Rice Brans

- T1=PRB(Raw)
- T2=PRB-I(20% moist and extruded)
- T3=PRB-II(20% moist with 1% solution of acetic acid and extruded)
- T4=PRB-III(20% moist with 1% solution of calcium hydroxide and extruded)
at 11–13% moisture content so moist heat is more effective to control the peroxidase. Later on Sayre et al. (1982) further revealed that steam injection i.e. 11 – 13% added moisture proved to be more beneficial non-specific proteinase. Lately Hammond (1994) established that 20% added moisture combined with acetic acid or calcium hydroxide and at higher temperature cooking (130±2 °C) of rice bran completely destroyed the peroxidase activity.

4.4.3 Haemagglutinin-lectin

Haemagglutinin-lectin are toxic globulin proteins concentrated in rice germ which is a part of rice bran, that when come in contact with blood agglutinate mammalian red blood cells (Ory et al., 1981; Tsuda, 1979; Benedito and Barber, 1978).

Results of haemagglutinin-lectin activity (HA) are shown in Table 8b. Haemagglutinin activity was reduced by 95% with cooking the moist rice bran at 130 ± 2 °C. Similar results were explored when moist acetic acid or calcium hydroxide (1% sol.) treated rice brans were extruded. Almost no activity was indicated in 20% moist with 1% sol. of acetic acid and extruded rice bran. It can be assumed that moist extrusion, moist acetic acid or Ca(OH)_2 treated and extruder cooked operation in rice bran inactivated all the HA activity leading to a conclusion that toxic globulin proteins were permanently denatured with moist heat, pressure, acid and alkali treatments given to rice bran.

The results are fully in conformity with the findings of Sayre et al., 1987, Benedito and Barber, 1978. Rehman and Mahmood (1996) further confirmed the present results who reported that by heating the rice bran at 100 °C up to six minutes haemagglutinin activity was inactivated. However, Peumans et al. (1983) concluded that there was no loss of haemagglutinin activity by heating the rice bran at 70 °C for 10 minutes with 0.2M NaCl, even some HA was reported at 100 °C for 10 minutes. Alvi et al. (1972)
Table 8b. Means for haemagglutinin-lectin, trypsin inhibitor activity and phytates of raw and processed rice brans

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Haemagglutinin-lectin activity</th>
<th>Trypsin inhibitor activity</th>
<th>Phytates %</th>
<th>Reduction in phytates %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>23.92</td>
<td>8018.00</td>
<td>4.25</td>
<td>-</td>
</tr>
<tr>
<td>T₂</td>
<td>0.96</td>
<td>0.00</td>
<td>1.95</td>
<td>54.12</td>
</tr>
<tr>
<td>T₃</td>
<td>0.01</td>
<td>0.00</td>
<td>0.45</td>
<td>89.88</td>
</tr>
<tr>
<td>T₄</td>
<td>0.10</td>
<td>0.00</td>
<td>0.95</td>
<td>77.65</td>
</tr>
</tbody>
</table>

T₁ = RRB (Raw)  
T₂ = PRB-I (20% moist and extruded)  
T₃ = PRB-II (20% moist with 1% sol. of acetic acid and extruded)  
T₄ = PRB-III (20% moist with 1% sol. of calcium hydroxide and extruded)
stated that co-extrusion, moist cooking with chemical treatment almost diminished the haemagglutinin activity.

4.4.4 Trypsin Inhibitor

Trypsin inhibitors are the most important anti-nutritive factors present in rice bran (Kratzer et al., 1974) but these are readily inactivated by moist heat treatment (Deolanker and Singh, 1979.)

The results of trypsin inhibitor activity (TIA) are indicated in Table 8b. By moist heat treatment, it was completely eradicated in PRB. The results of this study are partially in agreement with Rehman and Mahmood (1996) who reported that autoclaving rice bran at 121 °C (15psi) for 5-20 minutes indicated 10% reduction in TIA. The results of the present investigation are in accordance with earlier findings of Kratzer and Payne, (1977). Apart from temperature and stability of rice bran, the TIA was observed to be pH dependent (Tashiro and Maki, 1986). It was also inactivated by steaming of rice bran at 100 °C for 6 minutes.

4.4.5 Phytate

The largest part of phosphorous (P) in rice bran is linked to inositol in Ca-Mg salt of myoinositol hexaphosphite or phytin (Luh et al., 1991). Phosphate group of rice bran can readily form complexes with calcium (Ca), zinc (Zn) and iron (Fe) and also with protein (Juliano, 1985). As phytate P is relatively unavailable to non-ruminants so phytate may also render other minerals poorly available as reported by Warren and Farrell, 1990.

The effect of processing on phytate contents (%) of different rice brans is given in Table 8b. The phytate contents of rice bran (RB) 20% moist with extrusion was reduced by 54.12%. However the phytates in processed rice brans (moist with acetic acid and calcium hydroxide plus extruder cooking), were further decreased by 89.88 and 77.65%, respectively. The results of the present study are quite in concordance with
the findings of Tangendjaja and Lowary (1985) who reported that incubation of rice bran at 55°C reduced rice bran phytic acid content by 80% and Sayre et al. (1988) suggested the addition of Ca (10g/kg) to stabilize the rice bran diets for achieving the superior growth results in chicks. This was due to the reason that extrusion cooking enhanced the phosphorous availability by breaking the bonds between phytate and phosphorous releasing the phosphorous.

4.4.6 Microbial load, color and structure of rice brans

The microbial load on raw and processed rice brans during storage has been illustrated in Fig. 3 and 4. Bacterial count (total viable count) of raw rice bran (RRB) was observed to be 2.80 x 10^2 cfu/g, while the mould count was found to be 1.14 x 10^2 cfu/g after 90 days storage period (Table 9). It is clear from the results that with increasing storage time both bacterial and mould count were increased. Among the processed rice bran samples, at the initiation of the study calcium hydroxide treated sample had least value for microbial count including bacteria and mould while acetic acid treated samples showed better results at the end of study.

During the present investigation, it was concluded that extrusion cooking along with respective treatment had inverse relation with bacterial and mould counts. The results further depicted that bacterial and mould count remained lower in acetic acid treated rice bran (PRB-II) than calcium hydroxide treated rice bran (PRB-III). However, an increasing trend in both parameters was observed during storage period of 90 days.

The present results are in accordance to the findings of Plavnik and Sklam (1995) and Said (1996) who explored that due to the extrusion, rearrangement of chemical structure took place and nutrients are not available to micro organisms for their optimum growth.

The color of RRB remained brownish while color of PRB-II and PRB-III changed to off white after 90 days storage. The structure of raw rice
Fig. 3 Effect of Storage on Bacterial count of Rice Brans

- $T_1$ = PRB (Raw)
- $T_2$ = PRB-I (20% moist and extruded)
- $T_3$ = PRB-II (20% moist with 1% solution of acetic acid and extruded)
- $T_4$ = PRB-III (20% moist with 1% solution of calcium hydroxide and extruded)

Bacterial Count (1x100/g)

Days

0 30 60 90
Fig. 4 Effect of Storage on Mould count of Rice Brans

- T1=PRB(Raw)
- T2=PRB-I(20% moist and extruded)
- T3=PRB-II(20% moist with 1% solution of acetic acid and extruded)
- T4=PRB-III(20% moist with 1% solution of calcium hydroxide and extruded)
Table 9. Bacterial count, mould count, color and structure after 90 days of raw and processed rice brans

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Bacterial count/g</th>
<th>Mould count/g</th>
<th>Color</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>2.80x10²</td>
<td>1.14x10²</td>
<td>Brown</td>
<td>Fluffy powder</td>
</tr>
<tr>
<td>T₂</td>
<td>0.38x10²</td>
<td>0.63x10²</td>
<td>Lemon yellow</td>
<td>Crumble</td>
</tr>
<tr>
<td>T₃</td>
<td>0.21x10²</td>
<td>0.59x10²</td>
<td>Off white</td>
<td>Crumble</td>
</tr>
<tr>
<td>T₄</td>
<td>0.24x10²</td>
<td>0.61x10²</td>
<td>Off white</td>
<td>Crumble</td>
</tr>
</tbody>
</table>

T₁ = RRB (Raw)
T₂ = PRB-I (20% moist and extruded)
T₃ = PRB-II (20% moist with 1% sol. of acetic acid and extruded)
T₄ = PRB-III (20% moist with 1% sol. of calcium hydroxide and extruded)
bran samples showed fluffy appearance while the remaining samples had crumble look (Table 9). The change in color was due to the reason that all treated brans were extruded except raw rice bran (control). These findings are in agreement with the results reported by Jiaxun (2001) who reported that acid stabilized parboiled rice bran could maintain its stability up to 6 months storage at ambient conditions.

4.5 Chemical Composition of Bread

One of the objective of this study was to improve the nutritional value of bread by incorporation of various proportions of processed rice bran possessing minimum anti-nutritional factors to wheat flour. The effect of supplementation of PRB on chemical composition of breads i.e. crude protein, crude fat crude fiber, ash and NFE were studied.

The processed rice bran PRB-II (20% moisture with 1% acetic acid solution and extrusion) @ 5, 10, 15, 20, 25 and 30% was incorporated in wheat flour for the preparation of bread. The results obtained were tabulated and subjected to statistical analysis using analysis of variance techniques (Table 10).

The mean values for different chemical constituents have been presented in Table 11. The data revealed significant ($P \leq 0.01$) differences existed among various treatments in crude protein, crude fat, crude fiber, ash and NFE. This indicated that inclusion of processed rice bran ($T_1-T_7$) at various proportion to wheat flour affected the proximate composition of breads ($T_1-T_7$), which were significantly different among each other.

The comparison of means by using Duncan Multiple Range Test techniques in Table 11, showed that the protein content of bread, in which 30% of processed rice bran (PRB) was incorporated ($T_7$), showed significantly higher protein content (12.94%) than all the other breads ($T_1-T_6$), while $T_1$ showed significantly the lowest protein content (11.87%), where no PRB had been incorporated in bread. The addition of PRB to
Table 10. Mean squares for chemical composition of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>0.472**</td>
<td>10.715**</td>
<td>2.638**</td>
<td>0.945**</td>
<td>44.217**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.043</td>
<td>0.121</td>
<td>0.199</td>
<td>0.029</td>
<td>1.712</td>
</tr>
</tbody>
</table>

** = P ≤ 0.01
Table 11. Chemical composition of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Crude Protein %</th>
<th>Crude Fat %</th>
<th>Crude Fiber %</th>
<th>Ash %</th>
<th>NFE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>11.87e</td>
<td>3.64f</td>
<td>0.62e</td>
<td>1.52e</td>
<td>82.35a</td>
</tr>
<tr>
<td>T₂</td>
<td>12.08de</td>
<td>4.31e</td>
<td>0.88de</td>
<td>2.13de</td>
<td>80.60ab</td>
</tr>
<tr>
<td>T₃</td>
<td>12.29cd</td>
<td>5.47d</td>
<td>1.13cd</td>
<td>2.40d</td>
<td>78.71b</td>
</tr>
<tr>
<td>T₄</td>
<td>12.51bc</td>
<td>6.35c</td>
<td>1.39bc</td>
<td>2.85cd</td>
<td>76.90b</td>
</tr>
<tr>
<td>T₅</td>
<td>12.72ab</td>
<td>7.25b</td>
<td>1.65b</td>
<td>3.29bc</td>
<td>75.09b</td>
</tr>
<tr>
<td>T₆</td>
<td>12.81ab</td>
<td>8.14a</td>
<td>1.96a</td>
<td>3.76ab</td>
<td>73.33c</td>
</tr>
<tr>
<td>T₇</td>
<td>12.94a</td>
<td>8.63a</td>
<td>2.15a</td>
<td>4.18a</td>
<td>72.10c</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control
T₂ = 5% PRB
T₃ = 10% PRB
T₄ = 15% PRB
T₅ = 20% PRB
T₆ = 25% PRB
T₇ = 30% PRB
wheat flour increased the protein content from 11.87 to 12.94%. There were non-significant differences among T₅ (12.72%), T₆ (12.81%) and T₇ (12.94%) while T₄ (12.51%) was non-significant with T₃ (12.29%) and significantly different from T₂ (12.08%) and T₁ (11.87%) for protein content.

The mean values for crude fat of various treatments (T₁ – T₇) have been shown in Table 11. The addition of PRB to wheat flour increased the fat content of breads by 18.41, 50.27, 74.45, 99.18, 123.63 and 137.09%, respectively. Crude fat was significantly higher in T₇ and significantly lower in T₁ (Control). Comparison of mean values (Table 11) for crude fat showed a progressive increase in the fat content on supplementation of PRB to wheat flour. The fat content was statistically significant among all treatments except T₆ and T₇ where it was non-significant. This increase in fat content was due to higher amount of fat in PRB than wheat flour.

The crude fiber (CF) content of various breads has been shown in Table 11. The CF of bread increased from 0.62 to 2.15%. The comparison of mean values revealed statistically non-significant differences between T₆ & T₇ and T₁ & T₂.

Ash content of any food indicates the amount of minerals in it. The wheat flour contained 1.52% ash. On inclusion of PRB to wheat flour the ash content of breads increased (Table 11) which was significantly higher in T₇ and significantly lower in T₁.

Inclusion of PRB to wheat flour decreased the NFE of breads. Comparison of the mean values showed that amount of NFE was significantly higher in wheat flour (T₁) bread than the PRB supplemented breads. The breads containing 5,10,15 and 20% PRB showed statistically non-significant differences for NFE but these were significantly different from all other breads. The mean NFE values of T₆ and T₇ were also found to be non-significantly different.
The results of the present study are in line with the investigation of Taha et al. (1982) who worked on enrichment of wheat bread with rice bran. They concluded that protein content of bread increased significantly by the supplementation of rice bran to wheat flour as it is observed in the present study. Hussain (1985) reported that the protein content of pan bread was 8.3%. Later on Holland et al. (1991) reported that protein content was 7.6% in white sliced bread while it was 8.5% in brown bread so wheat bran increased the protein content of bread. Lynn (1969) reported that rice bran may be added at levels of 5 to 15% in various baked products (bread and cookies). It improved the lysine content of bread and gave a bland flavor and adds significant amount of amino acids, minerals and vitamins to baked goods. The present results are also in close resemblance to that of Sharma and Chauhan (2002) who concluded that addition of rice bran to wheat flour increased the contents of proteins and lysine proportionality to the level of substitution.

The results regarding crude fat resemble with the earlier findings of Hussain (1985) who reported that the crude fat in pan bread was 1.2%. Zia-ul-haq (2001) further supported the present investigation, who accomplished that commercial branded and unbranded breads contained fat contents from 1.50 to 1.60% (as such basis).

The results correlate with the findings of Hussain (1985) who determined that pan bread manufactured from white flour had 1.5% crude fiber. Which is an excellent source of dietary fiber as reported by Sultan (1969). Krishnan et al. (1987) substituted oat bran with wheat flour and concluded that CF increased significantly by its supplementation. Like wise Holland et al. (1991) demonstrated that crude fiber content of white and brown bread were 1.5 and 3.5%, respectively. So the fiber content of bread increased by the incorporation of rice bran. In the recent studies, Sharma and Chauhan (2002) substituted rice bran with wheat flour in proportions of
5-20% in breads and concluded that by the addition of rice bran to wheat flour increased the dietary fiber in proportion to the level of supplementation. The CF contents of present study showed that dietary fiber was quite in range required for health.

The results of the present study also favor the conclusions made by Holland et al. (1991) who investigated that wheat flour with 70% extraction had 0.44% ash as compared to 0.92% in 85% extraction. Ash contents could be improved by increasing degree of milling in rice bran i.e. brown to white. In a similar study, Akhtar (1993) found 1.11% ash content in 1% guar bread.

Hussain (1985) reported 51.5% NFE in pan bread. Later on Akhtar (1993) studied NFE (46.12%) in proximate composition of pan bread having 1% guar gum. The data was expressed on as such basis but in this study the results have been presented on dry basis which indicate some differences in results. The NFE found in the present studies is in the higher limits of Hussain (1985) and Akhtar (1993) which might be due to the incorporation of PRB.

4.6 Sensory Evaluation of Bread

4.6.1 External Characteristics of Bread

The data on sensory evaluation of external characteristics i.e. volume, color of crust, symmetry of form, evenness of bake and character of crust of experimental breads prepared by incorporation of PRB to wheat flour at levels of 0, 5, 10, 15, 20, 25 and 30% (T₁-T₇) were statistically analysed, using analysis of variance techniques. The results showed significant (P≤0.01) differences in the scores for volume, color of crust, symmetry of form and character of crust (Table 12). However, evenness of bake showed statistically non-significant differences among various PR breads (T₁-T₇).
Table 12. Mean squares for external characteristics of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Volume</th>
<th>Color of crust</th>
<th>Symmetry of form</th>
<th>Evenness of bake</th>
<th>Character of crust</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>1.099*</td>
<td>1.484**</td>
<td>1.525**</td>
<td>0.207&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.510*</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.319</td>
<td>0.150</td>
<td>0.316</td>
<td>0.259</td>
<td>0.491</td>
</tr>
</tbody>
</table>

* = P≤0.05  
** = P≥0.01  
NS = Non significant
Table 13. Means for external characteristics of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Volume</th>
<th>Color of crust</th>
<th>Symmetry of form</th>
<th>Evenness of bake</th>
<th>Character of crust</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_1 )</td>
<td>6.81c</td>
<td>5.47ab</td>
<td>7.47bcd</td>
<td>8.48a</td>
<td>6.79abc</td>
</tr>
<tr>
<td>( T_2 )</td>
<td>7.25b</td>
<td>5.98a</td>
<td>8.11abc</td>
<td>8.71a</td>
<td>7.38ab</td>
</tr>
<tr>
<td>( T_3 )</td>
<td>7.58b</td>
<td>5.81a</td>
<td>8.52ab</td>
<td>8.54a</td>
<td>7.56a</td>
</tr>
<tr>
<td>( T_4 )</td>
<td>8.31a</td>
<td>5.84a</td>
<td>8.78a</td>
<td>8.66a</td>
<td>7.69a</td>
</tr>
<tr>
<td>( T_5 )</td>
<td>7.58b</td>
<td>4.83bc</td>
<td>7.51bcd</td>
<td>8.11a</td>
<td>6.85abc</td>
</tr>
<tr>
<td>( T_6 )</td>
<td>6.72c</td>
<td>4.75cd</td>
<td>7.23cd</td>
<td>8.03a</td>
<td>6.13bc</td>
</tr>
<tr>
<td>( T_7 )</td>
<td>6.63c</td>
<td>4.11d</td>
<td>6.82d</td>
<td>8.01a</td>
<td>5.84c</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

\( T_1 = \) Control \hspace{1cm} \( T_5 = \) 20% PRB
\( T_2 = \) 5% PRB \hspace{1cm} \( T_6 = \) 25% PRB
\( T_3 = \) 10% PRB \hspace{1cm} \( T_7 = \) 30% PRB
\( T_4 = \) 15% PRB
The scores assigned to volume of bread ranged from 6.63 to 8.31 among different treatments. The data presented in Table 13 showed that significantly higher scores were given to volume of bread prepared from 15% PRB (T4) followed by T3, T5, and T2. The breads of treatments T2, T3 and T5 yielded statistically non-significant difference in volume. Similarly the scores for T1, T6 and T7 were also non-significant among each other. The scores given to volume of bread was significantly lower in T7 followed by T6 and T1.

The scores assigned to crust color of various breads ranged between 4.11 to 5.98 scores. The minimum scores for crust color were attained by the bread prepared from T7 (30% PRB) and maximum scores were given to bread of T2 (5% PRB). The scores for crust color were significantly higher of bread prepared from T2 followed by T4, T3 and T1, but these possessed non significant differences with each other. The scores for crust color was significantly lower in bread prepared from T7 followed by T6 but both of these treatments showed non significant difference for crust color.

The scores assigned by judges for symmetry of form ranged from 6.82 to 8.78 among breads of different treatments. The symmetry of form got significantly higher scores of bread prepared from T4 (15% PRB) followed by bread of T3 (10% PRB) and T2 (5% PRB). The differences in symmetry of form scores among these treatments were found to be non significant between breads of T3 and T2. However, significantly the lowest scores for symmetry of form were given to bread of T7 (30% PRB) followed by breads of T6 (25% PRB) and T5 (20% PRB). The differences in scores of symmetry of form of bread among these three treatments were not significantly different.

The scores for evenness of bake of bread were statistically non-significant among different breads prepared from various treatments,
indicating that incorporation of PRB up to 30% have no significant effect on evenness of bake of bread.

The scores given to breads for character of crust ranged from 5.84 to 7.69 scores (Table 13). While comparing the mean values for character of crust showed significantly the highest scores by bread of T4 but it showed non-significant differences with respect to scores given to character of crust of breads prepared from T1, T2, T3 and T5. The scores assigned to character of crust were significantly the lowest of bread prepared from treatment T7 followed by T6. This indicated that the character of crust was not affected to a great extent by incorporation of PRB to wheat flour upto 20%.

The volume of bread is an important parameter which determines its softness. It is a prime consideration in consumer acceptance. The greater the volume, the softer the bread loaf appears when squeezed. Its retention depends upon texture. During fermentation and proofing stages of bread manufacturing, fine cells of equal sizes proved to have the best texture. Ultimately this affects bread softness and volume.

In the present study, the findings are comparable to those reported by Sharp and Kitchen (1990) who found that rice bran can be replaced up to 15% in white flour breads. Later on Sharma and Chauhan (2002) substituted rice bran with wheat flour and concluded that at higher proportion of rice bran substitution, bread loaf volume was decreased and it is comparable with the present results which indicated that by addition of 25 and 30% rice bran, the loaf volume decreased as compared to control T1 where 100% wheat flour was used for bread preparation. The results of this study also resembles with the findings of Lima et al. (2002) who reported that functional properties of bread manufactured with supplementation of processed full fat rice bran for 10 and 20% replacement flour with PRB, the bread loaf volume increased 2% as
compared to control and decreased 6% but in this investigation, loaf volume increased maximum for 15% replacement of wheat flour with PRB and decreased to minimum for 30% substitution due to decrease in gluten of wheat flour which may have decreased the volume of bread.

During judging of baked bread, color of crust is very important criteria, which provides information about bread raw material, its formulation and quality. For good product appeal or marketing, golden brown crust color is liked by the consumers.

The results for color of crust are in concordance with the findings of Lynn (1969) who concluded that inactivated lipase in rice bran increased dough yield, contributed to an attractive tan crumb & crust. It did not disturb mixing or formation process of dough. By its substitution, baked products remained fresher with more moist condition. It also added amino acids, minerals and vitamins to baked goods.

The findings for symmetry of form, evenness of bake and character of crust are comparable to those reported by Taha et al. (1982) who worked on bread enrichment with rice bran and found that sensoric parameters were acceptable by its supplementation in wheat bread.

4.6.2 Internal Characteristics of Bread

The data on sensory parameters of internal characteristics (aroma, grain, color of crumb, taste and texture) of various breads prepared from wheat flour, supplemented with PRB, at 5, 10, 15, 20, 25 and 30% (T₁-T₇) levels were assessed by a panel of trained judges. The data were tabulated and analysis of variance techniques were applied and the results are given in Table 14. The results for aroma and color of crumb showed statistically significant differences at P ≤ 0.01 percent level among various treatments i.e. T₁ to T₇. Where as the grain size, taste and texture were statistically significant at P ≤ 0.05 percent level. This indicated that all the
internal characteristics based on sensory evaluation were significantly affected by the supplementation of wheat flour with PRB.

The results in Table 15 showed that scores assigned to crumb color of bread were significantly the highest when wheat flour was supplemented with 0, 5, 10 and 15% PRB. However, these treatments were statistically identical with respect to crumb color of bread. The breads prepared from wheat flour supplemented with 20, 25 and 30% were ranked at the bottom with respect to crumb color.

The scores assigned to aroma of bread ranged from 5.17 to 8.54 scores. The significantly higher scores were assigned to the bread prepared from wheat flour with 15% supplemented PRB (T₄) followed by 10% PRB (T₃) and 5% PRB (T₂). However, the differences among these treatments were non significant for aroma.

The scores assigned to aroma were significantly the lowest for the bread prepared from wheat flour supplemented with 30% PRB (T₇) followed by supplemented with 25% PRB (T₆). The differences between these two treatments were also found to be non significant.

The results in Table 15 manifested that grain of breads prepared from wheat flour supplemented with 0, 5, 10 and 15% PRB got significantly higher scores than other breads. However, the scores for grain did not differ significantly among these breads.

The taste of bread prepared from various PRB supplemented breads ranged from 5.24 to 7.14 scores. Significantly the highest scores for taste was assigned to the bread supplemented with 15% PRB (T₄) whereas significantly the lowest scores were assigned by the judges to the bread supplemented with 30% PRB (T₇) followed by 25% PRB (T₆) and 20% (T₅). The differences for taste of bread scores among T₁, T₂, T₃ and T₄ were found to be non significant with one another.
Table 14. Mean squares for internal characteristics of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Color of crumb</th>
<th>Aroma</th>
<th>Grain</th>
<th>Taste</th>
<th>Texture</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>4.668**</td>
<td>6.336**</td>
<td>2.983*</td>
<td>2.080*</td>
<td>4.419*</td>
<td>45.058**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.883</td>
<td>0.414</td>
<td>0.750</td>
<td>0.637</td>
<td>1.133</td>
<td>4.910</td>
</tr>
</tbody>
</table>

** = P≤0.01
*  = P≤0.05
Table 15. Means for internal characteristics of various processed rice bran supplemented breads

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color of crumb</th>
<th>Aroma</th>
<th>Grain</th>
<th>Taste</th>
<th>Texture</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>6.25ab</td>
<td>7.81a</td>
<td>8.17abc</td>
<td>6.18ab</td>
<td>8.13ab</td>
<td>71.56b</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.81a</td>
<td>8.03a</td>
<td>8.81ab</td>
<td>7.03a</td>
<td>8.55a</td>
<td>77.66a</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>7.42a</td>
<td>8.31a</td>
<td>8.93a</td>
<td>7.09a</td>
<td>8.97a</td>
<td>78.73a</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>7.31a</td>
<td>8.54a</td>
<td>9.11a</td>
<td>7.14a</td>
<td>8.92a</td>
<td>80.30a</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>5.15b</td>
<td>6.41b</td>
<td>7.23bc</td>
<td>5.84ab</td>
<td>6.73b</td>
<td>66.24c</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>5.02b</td>
<td>5.21c</td>
<td>6.95c</td>
<td>5.41b</td>
<td>6.64b</td>
<td>62.09c</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>4.95b</td>
<td>5.17c</td>
<td>6.78c</td>
<td>5.24b</td>
<td>6.55b</td>
<td>60.10d</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T<sub>1</sub> = Control  T<sub>5</sub> = 20% PRB
T<sub>2</sub> = 5% PRB  T<sub>6</sub> = 25% PRB
T<sub>3</sub> = 10% PRB  T<sub>7</sub> = 30% PRB
T<sub>4</sub> = 15% PRB
The texture of bread prepared from wheat flour supplemented with 0, 5, 10 and 15% PRB got significantly higher scores. The scores given to texture of breads prepared from wheat flour supplemented with 20, 25 and 30% PRB were ranked at bottom. However, all these three treatments were statistically identical to control (0% PRB) with respect to texture of bread.

The total scores of various breads (T₁ to T₇), evaluated by panel of judges is shown in Table 15. It is the summation of means for volume, color of crust, symmetry of form, evenness of bake, character of crust, aroma, grain, color of crumb, taste and texture.

The total bread scores (external and internal) evaluated subjectively showed that 15% PRB (T₄) supplemented bread ranked to the top (80.30) while 30% PRB (T₇) obtained the lowest scores (60.10).

As the PRB is normally finely granulated, light tan in color and possesses bland flavor with a nutty toasted overtone so its incorporation up to 15% in bread increased its sensoric attributes.

The total scores are used as a criteria for acceptance or rejection of breads i.e. decision making tool (DMT). On the basis of judgment made by panelists, the minimum score (accepted breads) was chosen for wheat flour bread (T₁) i.e. total score 71.56. The breads that scored lower than 71.56 were rejected. Addition of PRB to wheat flour increased the total scores by 8.5, 10.0, 12.2%, respectively by the supplementation of 5% PRB (T₂), 10% PRB (T₃) and 20% PRB (T₄) of wheat flour with PRB 5 (T₁), 10 (T₂) and 15% (T₃) (w/w). The maximum scores (80.30) were obtained by 15% PRB supplementation in wheat flour. There was non-significant differences among total scores of bread prepared from 20, 25 and 30% PRB supplemented wheat flour but these were ranked at bottom. It may be concluded from the results of external and internal characteristics assessment of breads by panel of judges that the breads prepared by the
supplementation of PRB up to 15% wheat flour level were scored better than control and other PRB supplemented level.

Krishnan et al (1987) studied the effects of oat bran supplementation (10-15 %) in bread. Dietary fiber and protein increased significantly with oat bran supplementation up to 10%. Breads with 10% oat bran had better loaf volume, grain and texture than 15%. Absorption requirements in dough’s increased with increase of bran levels. Aroma and taste of enriched rice bran bread increased with 2-acetyl-l-pyrroline (2AP) principal compound. Lima et al (2002) reported that addition of 10% full fat rice bran to the bread had no detrimental effect on texture but a very slight hardening of the loaves occurred with 20% full fat rice bran when compared to the control i.e. texture profile analysis observed non-significant difference as far as cohesiveness and springiness but bread hardens, gumminess and chewiness increased with increased levels of rice bran and was higher for defaulted rice bran bread than full fat rice bran bread. The results of the present study are in close agreement to the above workers especially with Lima et al. (2002) who reported that upto 10% full fat rice bran showed no detrimental effect on bread characteristics. In the present study it has been observed that processed rice bran upto 15% did not show any negative effect on bread scores but showed improvement in sensory parameters than control PRB bread.

4.7 Chemical Composition of Cookies

A thin crisp cake is made from fine white wheat flour (FWWF) and eggs some times spiced or mixed with currents, sweetened or unsweetened. It is made in many varieties of sizes, shapes and are very popular in the world. In England and Pakistan, it is called as biscuits but in USA named as cookies, contains 5% moisture. Its 60% weight is based on cereals. Some times decorated with non cereal products (cream, icing, jelly and jam etc). In decorated biscuits moisture is more than 5% due to
decorated ingredients. The flour used for cookies has 70–75% extraction rates (soft wheat) having more fluffy type flour with less damaged starch and low protein contents.

The cookies were prepared with the aim of improvement of nutrients (proteins, unsaturated fats, ash & minerals) by incorporation of PRB (0-30%) having less anti nutritional factors (PRB-II) to wheat flour. The cookies were evaluated by chemical analysis and sensory evaluation for acceptability.

The data regarding chemical composition of cookies was statistically analysed using analysis of various techniques. The results in Table 16 revealed existence of statistically significant differences (P ≤ 0.01) among cookies prepared from various PRB supplemented wheat flour.

The results for crude protein content of different cookies in Table 17 demonstrated that by supplementation of PRB at levels of 5, 10, 15, 20, 25 and 30% in wheat flour increased the protein content of cookies by 0.25, 0.48, 0.65 0.85, 1.08 and 1.30%, respectively. The highest protein content (9.40%) was found in cookies prepared from 30% PRB supplemented wheat flour in (T₇) where as the lowest (8.10%) was found in control (T₁) where no PRB was supplemented. When the mean values for protein were compared with each other, it indicated that the protein content in cookies of control (T₁) was found to be significantly the lowest protein than cookies of all other treatments. The cookies prepared from 5 (T₁) and 10% (T₂) PRB supplemented wheat flour were statistically identical with respect to protein content. Similarly protein in the cookies of 20, 25 and 30% PRB wheat flour (T₅, T₆ and T₇) showed statistically non-significant differences. It is evident that protein content increased progressively by the supplementation of any level of PRB in what flour. This increase was due to the incorporation of PRB which had 13.25% protein content.
Table 16. Mean squares for chemical composition of processed rice bran supplemented wheat flour cookies

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>6</td>
<td>0.552**</td>
<td>6.382**</td>
<td>2.349**</td>
<td>3.283**</td>
<td>40.535**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.028</td>
<td>0.618</td>
<td>0.0315</td>
<td>0.0199</td>
<td>4.417</td>
</tr>
</tbody>
</table>

** = P≤0.01
Table 17. Means for Chemical composition of various processed rice bran supplemented wheat flour cookies

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Crude Protein %</th>
<th>Crude Fat %</th>
<th>Crude Fiber %</th>
<th>Ash %</th>
<th>NFE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>8.10e</td>
<td>21.70e</td>
<td>0.30f</td>
<td>0.70f</td>
<td>69.20a</td>
</tr>
<tr>
<td>T₂</td>
<td>8.35de</td>
<td>22.35de</td>
<td>0.67e</td>
<td>1.13e</td>
<td>67.50ab</td>
</tr>
<tr>
<td>T₃</td>
<td>8.58cd</td>
<td>23.12cd</td>
<td>1.24d</td>
<td>1.65d</td>
<td>65.41bc</td>
</tr>
<tr>
<td>T₄</td>
<td>8.75bc</td>
<td>23.78bc</td>
<td>1.60c</td>
<td>2.11c</td>
<td>63.76c</td>
</tr>
<tr>
<td>T₅</td>
<td>8.95ab</td>
<td>24.53ab</td>
<td>1.95b</td>
<td>2.62b</td>
<td>61.95cd</td>
</tr>
<tr>
<td>T₆</td>
<td>9.18a</td>
<td>25.28a</td>
<td>2.34a</td>
<td>3.12a</td>
<td>60.08d</td>
</tr>
<tr>
<td>T₇</td>
<td>9.40a</td>
<td>25.51a</td>
<td>2.54a</td>
<td>3.63a</td>
<td>58.92d</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control  
T₂ = 5% PRB  
T₃ = 10% PRB  
T₄ = 15% PRB  
T₅ = 20% PRB  
T₆ = 25% PRB  
T₇ = 30% PRB
The results regarding crude fat content in cookies prepared by substituting wheat with PRB in different proportions is given in Table 17. The fat content in cookies prepared from control (T₁) was 21.70% (100% wheat flour), which gradually increased as the proportion of PRB increased in cookies. The supplementation of PRB to wheat increased the fat content of cookies from 21.70 to 25.51%. The comparison of mean values revealed that non-significant differences existed among the cookies prepared from 5 and 10% PRB supplemented wheat flour cookies (T₂ and T₃). Similarly the cookies prepared from 20, 25 and 30% PRB supplemented wheat flour (T₅, T₆ and T₇) were non-significantly different with respect to fat content. The increase in fat content was due to 14.17% fat of PRB supplemented in wheat flour.

Crude fiber is an insoluble organic residue which remains after acidic or alkaline treatment under certain conditions. The results regarding the fiber content of cookies given in Table 17 showed that the fiber content of cookies increased from 0.30 to 2.54% by the supplementation of PRB in wheat flour (T₁-T₇). The fiber content of cookies were statistically different from one another among T₁ to T₆ treatments, however the cookies prepared from T₆ & T₇ treatments showed no significant differences for fiber content.

Ash represents the mineral matter. The ash content of various cookies yielded similar trends as in case of fiber content was observed. The ash content of various treatments (T₁-T₇) in Table 17 revealed that the ash content ranged from 0.70-3.63% among cookies prepared from various PRB supplemented cookies.

The results regarding nitrogen free extract (NFE) showed that cookies of control (T₁) was found to be 69.20% which decreased 5 to 30% by supplementation of PRB in wheat flour of different treatments. The NFE ranged from 58.92 to 69.20% among various types of cookies. This
decrease can be attributed to high protein, fat, fiber and ash contents of PRB supplemented cookies than wheat flour cookies.

The results of the present study are in line with the findings of Lima et al. (2002) who worked on enrichment of bakery products with rice bran. He concluded that rice bran can be effectively utilized for human consumption, due to excellent neutracetical properties. The rice bran was processed by drum drying and pin milling for its incorporation in human diet. By this process, its hydration capacity was increased and grittiness removed by decreasing mean particle size from 444 to 72 mp.

The results of the present study are fully supported by Kennedy et al. (1996) who reported that rice bran could be added to various food products to the extent of 5–10%. The yields of the extrudate were increased by the blending of full fat rice bran but were decreased by the addition of defatted rice bran.

These results are also in concordance with Singh et al. (1995) who investigated that stabilized full fat rice bran up to 20% level and unstabilized full fat or stabilized defatted rice bran up to 10% was found suitable in various food products. In the recent studies, Sharma and Chauhan (2002) supplemented wheat flour with rice bran which was stabilized by dry heat and extrusion cooking at levels of 5 – 20% in cookies. They found that addition of rice bran to wheat flour increased the contents of protein and dietary fiber in cookies proportionality to the level of replacement as the increase for these parameters has been observed in the cookies prepared from PRB supplemented wheat flour.

The existing study is also fully in conformity with the findings of Chumachenko et al. (1987) who investigated that rice bran could be added from 5 – 25% in bakery products but it could not exceed 15% in flour containing low gluten.
Tangkanakal et al. (1995) reported that high fiber breads and biscuits made with 100% wheat flour had fiber contents of 3.06 and 2.70 g/100g respectively. Fiber contents of fiber enriched breads and cookies were considered acceptable with in the range of 7.4–13.4 g/100g and 1.17–20.82 g/100g respectively.

In the present study it may be assumed that the cookies prepared from PRB supplemented flour contain high protein with an improved level of lysine content which is deficient in cereals particularly wheat.

### 4.8 Sensory Evaluation of Cookies

The sensory evaluation is very important criteria in food industry which is usually performed at the end of the research and development cycle of the product. This attribute evaluated the ranking of a product on the basis of scores given by the panel of judges to the product. They rate like or dislike the various parameters. The cookies prepared from PRB (0-30%) supplement wheat flour were analyzed chemically and then the cookies were subjected to sensory evaluation for color, crispness, taste, texture and overall acceptability by a panel of trained judges for acceptance or rejection of the cookies. The results for sensory evaluation of cookies are discussed below.

The mean squares for different parameter based on sensory evaluation have been presented in Table 18. The results of the analysis of variance revealed that sensory scores assigned by judges for color, crispness, taste, texture and overall acceptability were found to be statistically significant. This indicated that addition of PRB in wheat flour exhibited marked effect on the sensory parameters of cookies.

The scores assigned by judges given in Table 19 indicated that the scores for color of cookies ranged from 4.21 to 7.81 scores among different treatments. The significantly higher scores were assigned to cookies prepared from 15% PRB (T₄) supplemented wheat flour.
Table 18. Mean squares for sensory evaluation of various processed rice bran supplemented wheat flour cookies

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Color</th>
<th>Crispness</th>
<th>Taste</th>
<th>Texture</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>5.120**</td>
<td>3.180**</td>
<td>3.210**</td>
<td>4.095**</td>
<td>35.846**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.472</td>
<td>0.226</td>
<td>0.129</td>
<td>0.183</td>
<td>3.018</td>
</tr>
</tbody>
</table>

** = P≤0.01
*  = P≤0.05
### Table 19. Means for sensory evaluation of processed rice bran supplemented wheat flour cookies

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color</th>
<th>Crispness</th>
<th>Taste</th>
<th>Texture</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>6.94ab</td>
<td>6.78a</td>
<td>6.54b</td>
<td>7.28b</td>
<td>27.54b</td>
</tr>
<tr>
<td>T₂</td>
<td>7.21a</td>
<td>6.84a</td>
<td>6.58b</td>
<td>7.11b</td>
<td>27.74b</td>
</tr>
<tr>
<td>T₃</td>
<td>7.54a</td>
<td>7.11a</td>
<td>7.11ab</td>
<td>8.82a</td>
<td>30.58a</td>
</tr>
<tr>
<td>T₄</td>
<td>7.81a</td>
<td>7.25a</td>
<td>7.37a</td>
<td>8.95a</td>
<td>31.38a</td>
</tr>
<tr>
<td>T₅</td>
<td>5.81bc</td>
<td>5.54b</td>
<td>5.58c</td>
<td>6.18c</td>
<td>23.11c</td>
</tr>
<tr>
<td>T₆</td>
<td>5.47c</td>
<td>5.28bc</td>
<td>5.25cd</td>
<td>6.24c</td>
<td>22.24c</td>
</tr>
<tr>
<td>T₇</td>
<td>4.21d</td>
<td>4.65c</td>
<td>4.89d</td>
<td>6.11c</td>
<td>19.86d</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control  
T₂ = 5% PRB  
T₃ = 10% PRB  
T₄ = 15% PRB  
T₅ = 20% PRB  
T₆ = 25% PRB  
T₇ = 30% PRB
The scores assigned to color were lower of cookies when prepared from T₅, T₆ and T₇ (20% or more PRB supplemented wheat). The differences in scores for color were found to be non significant when prepared from 0, 5, 10 and 15% PRB supplemented wheat flour (T₁, T₂, T₃ and T₄).

Statistical analysis for crispness indicated non-significant differences among scores of cookies prepared from 0, 5, 10 and 15% PRB supplemented wheat flour i.e. treatments T₁, T₂, T₃ and T₄. The scores assigned to crispness were also statistically non-significant between cookies prepared from 25 and 30% PRB supplemented wheat flour. The cookies prepared from 15% PRB wheat flour was preferred by the judges because it got maximum score (7.25).

Taste is a sense perceived by the tongue. Aroma and taste are closely allied characteristics. Taste is influenced by texture, flavor and composition of the product. It is one of the important factors in food preparation. The mean scores assigned to taste of cookies have been presented in Table 19. The scores assigned to taste of cookies prepared from 100% wheat flour was 6.54 which increased when 5, 10 and 15% PRB was supplemented in wheat flour. There was a maximum improvement in taste when cookies were prepared from 15% PRB supplemented wheat flour. The statistical analysis indicated non-significant differences among scores of cookies prepared from 0, 5 and 15% PRB supplemented wheat flour i.e. T₁, T₂ and T₃ treatments. The scores given to taste decreased when the level of PRB increased 20% or beyond 25 and 30%. The differences between T₆ & T₇ scores for taste of cookies was found to be non-significant.

The International standard organization (ISO) has defined texture as all the rheological and structural parameters of the food perceptible by means of mechanical, tactile and where appropriate, visual and auditory
receptors (ISO, 1981). Texture is also used as an indicator of food quality by the consumers. The results in Table 19 showed significant effect of the supplementation of PRB to texture of cookies. The scores assigned to cookies were significantly higher when prepared from 15% PRB supplemented wheat flour (T₄). There were non-significant differences in scores of cookies prepared from 0 & 5, 10 & 15, 20 & 25% of T₁ & T₂, T₃ & T₄ and T₅ & T₆ treatments. The results regarding texture scores indicated that texture of cookies improved by 15% PRB supplementation to wheat flour. However, the scores given to texture decreased when beyond 15% PRB was supplemented to wheat flour.

The criteria of total scores for acceptance or rejection of a product by panel of judges is based upon color, crispness, taste and texture. The total scores were calculated by the summation of scores assigned to all the above discussed sensory attributes and the results are shown in Table 19. The minimum total scores (19.86) were assigned to cookies prepared from 30% PRB supplemented wheat flour. The results based on total score showed that cookies prepared from 5, 10 and 15% PRB supplemented wheat flour got higher scores whereas the cookies prepared from 20, 25 and 30% PRB supplemented wheat flour were ranked at the bottom. It may be concluded that cookies prepared from 15% PRB (T₄) supplementation in wheat acquired the highest total scores (31.38) followed by T₃ (10% PRB) and T₂ (5% PRB) which attained 30.58 and 27.74 scores, respectively. The total scores showed non-significant differences between the cookies prepared from 10 and 15% PRB wheat flour. It may be recommended on the basis of results pertaining to sensory attributes that 15% PRB should be incorporated in wheat flour for the preparation of high quality cookies.

Sekhon et al. (1997) observed the effect of substituting the commercially available full fat rice brans (stabilized/parboiled) to wheat
flour in different food products. Muffin volume increased with the addition of rice bran to wheat flour but biscuits spread factor was not affected by the blending of full fat rice bran. However rice bran could be supplemented to different food products to the extent of 5–10%. Similar pattern was reported by Singh et al. (2000) who developed extruded crisp snacks from blends of wheat bran (up to 25%) and rice brackens. Sensory evaluation indicated that the incorporation of wheat bran up to 15% to rice brackens gave the products with the highest sensory scores. However, it was found that wheat bran may be included up to 25% in rice brackens without affecting sensory characteristics. As rice bran is rich in nutrients like wheat bran, having 15-20% rice tips (starch) so it can be incorporated in foods for increasing its neutraceutical and sensory attributes.

The results pertaining to sensory evaluation of cookies are fully in conformity with the findings mentioned above. As in the prevailing study lower scores were assigned to above 15% supplemented cookies by the trained panel of Judges. Types of bran also influenced cookies quality but effects varied with the level of addition. Thus the present results are identical with the findings of the above mentioned workers who reported that rice bran can be used upto 25% for the preparation of cookies.

4.9 Proximate Composition of Chapaties

The chapaties were prepared from whole wheat flour (100% extraction) alone and supplemented with PRB having less toxic factors (PRB-II) at levels of 0, 5, 10, 15, 20, 25 and 30% (T1-T7). The chapaties were prepared in a traditional way and nutritionally evaluated by:

1. Chemical analysis i.e. effect of PRB supplementation to whole wheat flour (WWF) on the chemical composition (crude protein, crude fat, crude fiber, ash and nitrogen free extract) of chapaties.
2. Subjective evaluation of sensory attributes of chapatis (color, flavor, taste, texture, feel to touch, foldability and breakability) by a panel of judges.

3. The chapatis accepted by the panel of judges were biologically evaluated by feeding young albino rats.

The statistical results regarding chemical analysis i.e. crude protein, crude fat, crude fiber, ash and NFE are presented in Table 20. The analysis of variance results revealed statistically significant \((P \leq 0.01)\) differences in chemical constituents such as crude protein, crude fat, crude fiber, ash and NFE among chapatis prepared from various processed rice bran supplemented whole wheat flour.

The results pertaining to crude protein presented in Table 21 showed that protein content showed non-significant differences among chapatis prepared from 0, 5, and 10% PRB supplemented whole wheat flour. However, \(T_4, T_5, T_6\) and \(T_7\) chapatis prepared from 15, 20, 25 and 30% PRB supplemented whole wheat flour \((T_4\) to \(T_7)\) also showed non-significant differences. The protein contents of chapatis increase from 11.23 to 12.83\% when prepared from 30\% PRB supplemented whole wheat flour. The crude protein was significantly higher in chapatis prepared from 30\% PRB supplemented whole wheat flour \((T_7)\) and significantly lower in chapatis prepared from 100\% whole wheat flour \((T_1)\).

There was an increase in the fat contents of chapatis when whole wheat flour was supplemented with PRB. This increase in fat contents may be due to the presence of higher amount of fat content in PRB i.e. 13.25\% as compared to 1.98\% fat in whole wheat flour. The addition of PRB increased the nutritive value of chapatis. The mean values for fat contents of all the chapatis differed significantly with one another.

The crude fiber content of chapatis increased from 2.11 to 3.79\% by the addition of processed rice bran to whole wheat flour. This increase
Table 20. Mean squares for chemical composition of chapatties of wheat flour supplemented with processed rice bran

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>1.533**</td>
<td>22.963**</td>
<td>1.026**</td>
<td>5.634**</td>
<td>106.166**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>0.311</td>
<td>0.307</td>
<td>0.022</td>
<td>0.018</td>
<td>5.920</td>
</tr>
</tbody>
</table>

** = P≤0.01
Table 21. Means for chemical composition of chapaties of wheat flour supplemented with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>T₁</td>
<td>11.23c</td>
<td>1.98g</td>
<td>2.11f</td>
<td>1.41f</td>
<td>83.27a</td>
</tr>
<tr>
<td>T₂</td>
<td>11.38c</td>
<td>3.11f</td>
<td>2.38e</td>
<td>2.16e</td>
<td>80.97ab'</td>
</tr>
<tr>
<td>T₃</td>
<td>11.63bc</td>
<td>4.48e</td>
<td>2.77d</td>
<td>2.67d</td>
<td>78.45bc</td>
</tr>
<tr>
<td>T₄</td>
<td>11.84ab</td>
<td>5.75d</td>
<td>2.93cd</td>
<td>3.85c</td>
<td>75.63cd</td>
</tr>
<tr>
<td>T₅</td>
<td>12.43ab</td>
<td>6.96c</td>
<td>3.18bc</td>
<td>3.92c</td>
<td>73.51de</td>
</tr>
<tr>
<td>T₆</td>
<td>12.56ab</td>
<td>8.26b</td>
<td>3.43b</td>
<td>4.62b</td>
<td>71.13ef</td>
</tr>
<tr>
<td>T₇</td>
<td>12.83a</td>
<td>9.47a</td>
<td>3.79a</td>
<td>5.21a</td>
<td>68.70f</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different.

T₁ = Control  
T₂ = 5% PRB  
T₃ = 10% PRB  
T₄ = 15% PRB  
T₅ = 20% PRB  
T₆ = 25% PRB  
T₇ = 30% PRB
was within the limit of nutritional values. The comparison of mean values, showed a statistically significant differences among crude fiber content of various chapatis. The increase in crude fiber contents of chapatis may be attributed to higher amount of crude fiber present in processed rice bran PRB than in whole wheat flour.

The ash content of chapatis prepared from various processed rice bran (T₁-T₇) have been shown in Table 21. The ash content increased progressively by the addition of PRB in whole wheat flour. The comparison of mean values of ash contents showed significant differences among various chapatis except chapatis prepared from 15 and 20% PRB supplemented whole wheat flour (T₄ and T₅) which were not significantly different for ash content. The ash content were significantly higher in T₇ and it was found to be significantly lower in chapatis prepared from 100% whole wheat flour (T₁).

The NFE content decreased with the addition of PRB to whole wheat flour for the preparation of chapati. The decrease may be attributed to the fact that PRB contained more protein, fat, fiber and mineral contents which decreased NFE parameters when PRB was supplemented in whole wheat flour.

The results for proximate composition of whole wheat flour were in accordance with Nagi et al. (1984) who found protein 9.45 – 11.41%, ash 16.5 – 1.67%. Similarly protein 10.60% and ash 1.50% in whole wheat flour was observed by Leelavathi et al. (1984). Likewise Tanaja et al. (1983) analysed four Indian wheat varieties and reported crude protein, crude fat, crude fiber and ash ranged between 10.11-11.77%, 1.74 – 2.93%, 0.98 – 1.42% and 1.43 – 1.79% respectively. The above results are somewhat in accordance with the present study. The whole wheat flour generally contained protein 12.2%, fat 2.3%, fiber 2.0% and carbohydrates
69% (FAO, 1989). Iftikhar (2002) reported that wheat flour contained protein 10.29%, fat 1.21% and NFE 76.71%.

So the chapatis with PRB supplementation had higher crude protein, crude fat, crude fiber and ash but lower NFE as compared with the chapatis of wheat flour only

4.10 Sensory Evaluation of Chapatis

Chapati is a staple diet of South Asian countries. A light brown and creamy color chapati is liked by the consumers.

The statistical results given in Table 22 indicated differences in flavor, taste, feel to touch, color, foldability and brakeability of chapatis prepared from various PRB supplemented whole wheat flours.

The judges assigned scores to color of chapatis higher when prepared from 5, 10 and 15% PRB supplemented whole wheat flour (T₂, T₃ and T₄). However, lower scores were assigned to color of chapatis when prepared from 20, 25 and 30% PRB supplemented whole wheat flour (T₅ to T₇). Its evident from Table 23 that the color scores assigned to chapatis were not significantly different in chapatis prepared from 0, 5, 10, 15, 20 and 25% PRB supplemented whole wheat flour (T₁ to T₆). The chapatis scores prepared from 30% PRB supplemented whole wheat flour was significantly the lowest for color attribute than the chapatis prepared from 5, 10 and 15% PRB chapatis. This indicated that addition of PRB to whole wheat flour did not show any significant influence on color of chapatis.

Higher scores were assigned to flavor of chapatis prepared with the addition of PRB to whole wheat flour @ 10 and 15% (T₃ and T₄). The scores assigned to chapatis prepared from 20, 25 and 30% PRB supplemented whole wheat flour were 6.54, 6.18 and 5.17. The differences in scores for flavor of chapatis of 10 and 15% PRB supplemented whole wheat flour chapatis were non-significant. Similarly chapatis of 5, 20 and
Table 22. Mean squares for various sensory attributes of chapatis of wheat flour supplemented with processed rice bran

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Total Scores</th>
<th>Breakability</th>
<th>Foldability</th>
<th>Feel to touch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>67.378**</td>
<td>1.201*</td>
<td>0.873*</td>
<td>1.084**</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Total Scores</th>
<th>Flavor</th>
<th>Taste</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>6</td>
<td>5.684**</td>
<td>3.145**</td>
<td>1.046*</td>
<td>0.721*</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td></td>
<td>0.309</td>
<td>0.145</td>
<td>0.243</td>
</tr>
</tbody>
</table>

P≤0.05
P≤0.01

*   **
Table 23. Means for various sensory attributes of chapaties of wheat flour supplemented with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>color</th>
<th>Flavor</th>
<th>Taste</th>
<th>Texture</th>
<th>Feel to touch</th>
<th>Fold - ability</th>
<th>Break - ability</th>
<th>Total Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>7.41ab</td>
<td>6.25b</td>
<td>7.81b</td>
<td>6.78a</td>
<td>7.15a</td>
<td>7.33ab</td>
<td>6.81a</td>
<td>49.54a</td>
</tr>
<tr>
<td>T₂</td>
<td>7.53a</td>
<td>7.13b</td>
<td>8.25ab</td>
<td>6.81a</td>
<td>7.50a</td>
<td>7.65a</td>
<td>6.23ab</td>
<td>51.10a</td>
</tr>
<tr>
<td>T₃</td>
<td>7.88a</td>
<td>8.11a</td>
<td>8.50ab</td>
<td>6.75a</td>
<td>7.32a</td>
<td>7.81a</td>
<td>6.18ab</td>
<td>52.55a</td>
</tr>
<tr>
<td>T₄</td>
<td>8.07a</td>
<td>8.75a</td>
<td>8.80a</td>
<td>6.91a</td>
<td>7.27a</td>
<td>7.35ab</td>
<td>6.75a</td>
<td>53.90a</td>
</tr>
<tr>
<td>T₅</td>
<td>7.40ab</td>
<td>6.54b</td>
<td>7.19b</td>
<td>5.75b</td>
<td>6.25b</td>
<td>6.88b</td>
<td>6.00abc</td>
<td>46.01b</td>
</tr>
<tr>
<td>T₆</td>
<td>7.28ab</td>
<td>6.18b</td>
<td>6.53c</td>
<td>5.64b</td>
<td>6.11b</td>
<td>6.51b</td>
<td>5.32bc</td>
<td>43.57b</td>
</tr>
<tr>
<td>T₇</td>
<td>6.52b</td>
<td>5.17c</td>
<td>6.14c</td>
<td>5.54b</td>
<td>6.04b</td>
<td>6.42b</td>
<td>5.18c</td>
<td>41.01b</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

\[ T₁ = \text{Control} \quad T₅ = 20\% \text{ PRB} \]
\[ T₂ = 5\% \text{ PRB} \quad T₆ = 25\% \text{ PRB} \]
\[ T₃ = 10\% \text{ PRB} \quad T₇ = 30\% \text{ PRB} \]
\[ T₄ = 15\% \text{ PRB} \]
25% PRB supplemented whole wheat flour were found to have non-significant with each other.

The taste score improved by 5.6, 8.8 and 12.7% in the chapaties prepared from 5, 10 and 15% PRB supplementation in whole wheat flour, respectively. The scores for taste of chapaties prepared from 0, 5, 10 and 20% (T₁, T₂, T₃ and T₅) differed non-significantly where as taste for chapaties of T₆ and T₇ treatments were also found to be non significant. The highest scores for taste was attained by T₄ followed by T₃ and T₂. The chapaties of 30% PRB supplemented in whole wheat flour got significantly the lowest scores for taste.

The scores for texture of various chapaties (T₁ to T₇) prepared by addition of PRB in whole wheat flour have been presented in Table 23. The scores given to chapaties prepared from 0, 5, 10 and 15% PRB whole wheat flour showed non-significant differences among one another. The scores for texture of chapaties of T₅, T₆ and T₇ treatments were also statistically non-significant. The scores for texture was significantly higher for chapaties supplemented with15% PRB in whole wheat flour (T₄) followed by 0, 5 and 10% PRB whole wheat flour (T₁, T₂ and T₃) whereas significantly lower scores were given to texture of chapaties prepared from T₇ followed by T₆ and T₅ treatment.

Feel to touch followed the same pattern of texture. The existence of comparison of mean scores (Table 23) by statistical analysis indicated non-significant differences in feel to touch among scores of chapaties prepared from 0, 5, 10 and 15 PRB whole wheat flour (T₁, T₂, T₃ and T₄). The scores assigned to chapaties of T₅, T₆ and T₇ treatments were also found to be non-significant. The higher scores for feel to touch were assigned to chapaties of T₂ followed by T₃, T₄ and T₁, where as the lowest scores were assigned by the judges to chapaties of T₇ followed by T₅ and T₆.
In case of foldability of chapaties, the scores assigned by judges ranged from 6.42 to 7.88 scores. The judges assigned significantly higher scores to chapaties prepared from 15% PRB whole wheat flour followed by 10, 15 and 20% PRB supplemented whole wheat flour. The significantly lower scores were attained by the chapaties of T7 followed by T6 and T5 treatments. The comparison of mean scores indicated that chapaties prepared from T1, T2, T3 and T4 treatment were found to possess non significant differences for foldability of chapaties. The scores assigned to chapaties of T1, T4, T5, T6 and T7 treatments was also found to be non significant.

The scores for breakability of chapati ranged from 5.18 to 6.81 scores among different treatments. The scores for breadability of chapaties for T1 was significantly higher followed by T4, T2 and T3 but the differences among these chapaties for this trait were non significant. Significantly the lowest scores to breakability were given to chapaties of T7 followed by T6 and T5 treatments.

Total scores (TS) of various chapaties (color + flavor + taste + texture + feel to touch + foldability + breakability) have been presented in Table 23. The chapaties prepared from PRB supplemented at levels of 5, 10 and 15% increased progressively for the total scores assigned by judges. Treatments T5, T6 and T7 obtained lower total scores than whole wheat flour chapaties and their mean scores showed statistically non significant differences with one another. On the basis of the above results, the chapaties prepared from 0, 5, 10 and 15% (T1, T2, T3 and T4) were more accepted by the panel of judges than chapaties prepared from T5, T6 and T7 treatments.

The total scores of sensory evaluation of chapaties were carried by a trained taste panelists. These were scored on Hedonic score system pertaining to the method of Meilgaard (1991) to find out the best
supplementation of PRB to WWF for its commercialization. The results revealed that chapaties were acceptable based on scores containing PRB upto 15% in whole wheat flour. The chapaties prepared from whole wheat flour containing PRB more than 15% showed a decline in overall scores in chapaties.

The results are fully supported by Kennedy et al. (1996) substituted two rice fractions, rice bran milk and rice bran fiber to replace margarine and flour from bakery products. Color become lighter as more margarine was replaced and darker with RBF addition. Tenderness increased with RBM and decreased with RBF as in this study these decreased above 15% replacement. Likewise Prakash (1996) concluded that incorporation of roasted rice bran (5–20%) in traditional foods had most impact on color but some impact on aroma, taste and overall acceptability and little effect on texture. It might be due to inhibition of FFA in full fat rice bran. Lima et al. (2002) indicated that full fat rice bran supplementation in bakery products gave better texture, showed no detrimental effect of 10% full fat rice bran and a very slight hardening of loaves with 20% full fat rice bran.

The present results are in accordance with the findings of Sekhon et al. (1997) who reported that the yields of extrudate were increased by the blending of full fat rice bran but were decreased by the addition of defatted rice bran. In different food products, rice bran could be added to the extent of 5–10%. However, the full fat rice bran could not be used for the production of extruded snack food. It might be due to its high fiber contents.

The results of the present study suggest that processed rice bran can be incorporated in whole wheat flour for the preparation of chapaties. The chapaties made from this flour will not only possesses better sensory attributes but also have better nutritional quality.
4.11 Biological Evaluation of Chapaties

Two feeding trials were conducted by feeding chapaties to young albino rats to determine weight gain, feed consumed, protein efficiency ratio (PER), true digestibility (TD), biological value (BV) and net protein utilization (NPU) of chapaties prepared by supplementation of PRB to whole wheat flour (WWF).

The data on biological parameters have been tabulated and subjected to statistical analysis, using analysis of variance techniques. The results of analysis of variance (ANOVA) in Table 24 revealed statistically significant ($P \leq 0.01$) difference for weight gain, feed consumed, PER, TD, BV and NPU values of various PRB supplemented whole wheat flour chapaties.

The weight gain of rats fed on chapaties ($T_1-T_5$) has been illustrated in Table 25. The data regarding weight gain showed that rats fed casein diet gained maximum weight (83g) as compared to other diets. The mean weight gain values when compared statistically indicated that casein diet and diet of $T_5$ in which 15% PRB was added in WWF showed statistically non-significant differences with each other. Similarly the weight gain of chapaties prepared from 5 and 10% PRB supplemented WWF were also statistically non-significant with each other. The rats fed on 100% whole wheat flour chapaties possessed the lowest weight gain (63g) but diet of chapaties containing 15% PRB supplemented to WWF showed the highest gain in weight i.e. 82g. It is evident from the results that supplementation of wheat flour with 15% PRB increased the weight gain of rats comparable to the weight gain of casein diet.

The average feed intake (feed consumed) of different groups of rats is shown in Table 25. The rats with 15% PRB supplemented WWF chapaties consumed more feed as compared to all other PRB supplemented WWF chapaties. The comparison of means for feed intake
Table 24. Mean squares for various nutritional attributes in different groups of albino rats fed on various experimental diets prepared from various PRB supplemented chapaties

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Wt. gain/group</th>
<th>Feed consumed/group</th>
<th>Protein efficiency ratio</th>
<th>True digestibility</th>
<th>Net protein utilization</th>
<th>Biological value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>292.972**</td>
<td>2555.40**</td>
<td>0.575**</td>
<td>36.900**</td>
<td>258.900**</td>
<td>232.500**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>30.359</td>
<td>232.62</td>
<td>0.006</td>
<td>9.087</td>
<td>5.794</td>
<td>6.859</td>
</tr>
</tbody>
</table>

** = P≤0.01
Table 25. Means for various nutritional attributes in different groups of albino rats fed on various experimental diets prepared from various PRB supplemented chapaties

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Wt. gain/group (g)</th>
<th>Feed intake/group (10 days) (g)</th>
<th>Protein efficiency ratio</th>
<th>True digestibility %</th>
<th>Net protein utilization %</th>
<th>Biological value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>83a</td>
<td>329c</td>
<td>2.53a</td>
<td>94a</td>
<td>73a</td>
<td>78a</td>
</tr>
<tr>
<td>T₂</td>
<td>63c</td>
<td>385c</td>
<td>1.30c</td>
<td>85b</td>
<td>49d</td>
<td>54c</td>
</tr>
<tr>
<td>T₃</td>
<td>70b</td>
<td>388b</td>
<td>1.81b</td>
<td>86b</td>
<td>54c</td>
<td>61c</td>
</tr>
<tr>
<td>T₄</td>
<td>73b</td>
<td>391b</td>
<td>1.87b</td>
<td>88ab</td>
<td>58c</td>
<td>65bc</td>
</tr>
<tr>
<td>T₅</td>
<td>82a</td>
<td>423a</td>
<td>1.94b</td>
<td>89ab</td>
<td>64b</td>
<td>67b</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Casein  
T₂ = Control  
T₃ = 5% PRB  
T₄ = 10% PRB  
T₅ = 15% PRB
showed existence of non-significant differences between casein diet ($T_1$) and chapatties of 5% PRB supplemented WWF ($T_2$). The results indicated that supplementation of chapatties with PRB increased the palatability of chapatties.

The PER values for casein found to be 2.53 and for chapatties of 100% whole wheat flour was found to be 1.30. The chapatties prepared from supplementation of 5, 10 and 15% PRB increased the PER values by 39, 44 and 49%, respectively. These PER values were non-significantly different with one another (Table 25).

The true digestibility (TD) of various diets ($T_1$ to $T_5$) are shown in Table 25. The TD for casein was observed to be maximum i.e. 94%. The TD was non-significantly different with diet of chapatties containing the 10 and 15% PRB in WWF ($T_4$ and $T_5$). The TD of chapatties prepared from 100% whole wheat flour and 5% PRB supplemented WWF was statistically at par for this biological parameter.

The net protein utilization (NPU) of whole wheat flour chapatties increased with the addition of PRB up to 15% level in whole wheat flour. The NPU was maximum (64%) for chapatties prepared from 15% PRB WWF ($T_5$). It may be concluded on the basis of the above findings that nutritive value of wheat flour improved by the supplementation of PRB to whole wheat flour up to 15% level on w/w basis. The best response in terms of chemical analysis, sensory and biological evaluation was also obtained with 15% PRB addition to whole wheat flour for the preparation of various chapatties.

The biological values of various diets are given in Table 25. The casein diet showed a maximum BV of 78%. The BV of chapatties of 100% whole wheat flour was found to be 54%. The BV of chapatties increased due to addition of 5, 10 and 15% PRB to whole wheat flour ($T_3$, $T_4$ and $T_5$).
by 13, 20 and 24%, respectively. Therefore, the addition of PRB in whole wheat flour improved the BV of chapatties.

The results of the feeding trial of rats confirmed that toxic factors of rice bran were removed on processing as the feeding values of PRB (5–15%) were improved as compared to control (0% PRB) and it could be served as a stuff because it had better amino acids profile than wheat particularly lysine and methionine which are limiting in wheat flour (Saunders, 1990). The improvement in weight gain, feed consumption, PER, TD, BV and NPU of rats fed chapatties prepared from PRB supplemented whole wheat flour are certainly due to high nutritional value of rice bran protein and better quantity and quality of other constituents such as fat in rice bran (Sharma 2002; Luh et al. 1991; Saunders 1990; Nicolosi 1989; Gerhard and Gallo 1989; Hegsted et al., 1990). The minerals and vitamins present in rice bran also complemented the wheat flour chapatties enriched with PRB (Saunders 1990 and Idouraine et al. 1986). The processing of rice bran improved its nutritional value (Farrell 1994, Dale 1997, Sayre et al. 1987) thus unlocking the toxins complex with proteins, minerals (Phytate) and carbohydrates. The results of the study are of great impact on national economy and health. By sparing 15% wheat from chapati and supplementing it with 15% PRB, we can increase the nutritional quality of our daily diet.

The results of the present study are fully in concordance with Prakash and Ramantham (1995) who observed that rice bran proteins (16.5–18.2%) were rich in albumins (32%) and globulins (26%). The PER of acid stabilized rice bran (RB) was ranked to the top (2.18) followed by untreated (2.09), heat stabilized (2.03) and parboiled (1.99) rice bran. They further reported that acid stabilized RB did not influence protein quality in food products. PER of rice bran ranged from 1.6 to 1.9 as compared to casein value of 2.5 while protein concentrate extracted from rice bran,
values from 2.0 to 2.5 had been observed in the previous studies. These findings are in agreement with the present investigations. The digestibility of rice bran protein is 73% where as its concentrate more than 90% (Saunder, 1990 and Sayre et al., 1987). These observations are also in accordance with the present research work. The processing of rice bran inactivated or eliminated the toxic factors which hindered the digestibility and availed ability of nutrients. The digestibility of wheat flour had been reported in the range of 67 – 94% by various researchers (Gilani et al. 1986, Chandra and Ramanatham, 1987). This difference might be due to protein intake or method of diet preparation or quality of whole wheat flour used. Above results are in agreement with the present efficacy study. The biological value of PRB (5–15%) supplemented diets had been improved as compared with control, indicating an improvement in whole wheat flour protein retention due to PRB supplementation. It might be due to improvement of limiting amino acids (Saunders, 1990).

The results indicated that better growth (weight gain) and feed efficiency also showed better PER, TD, BV and NPU, weight gain was correlated with PER (Zombade and Ichhponani 1983). Protein concentrate of rice bran had better digestibility and NPU than other cereal proteins (Ledesma et al. 1990). By the processing of rice bran, hydrolysis of protein occurred by liberating amino acids which become available to the rats. Ultimately PER and BV were improved in the PRB supplemented chapaties. It may be concluded from this research work that incorporation of PRB (5–15%) may prove to be beneficial for growth, feed consumption, PER, TD, BV and NPU in rats fed on experimental diets prepared from chapaties.

The results of the present study are supported by Sekhon et al., (1997) who found that by blending of full fat and defatted rice bran in various food products, the yields of extrudate increased by the blending of
full fat rice bran but were decreased by the addition of defatted rice bran. However rice brans (5 – 10%) could be added to different food products.

Singh et al. (1995) indicated that stabilized full fat rice bran could be used up to 20% level in various food products. It resembles to the present study where 5– 30% full fat rice bran had been used in the preparation of chapatties. Tashiro and Maki (1986) discussed that protein concentrate of defatted rice bran contained 45% protein which was a good source as casein in terms of protein quality being judged from amino acids analysis. Lima et al. (2002) reported that rice bran could be effectively utilized (10-20%) for human consumption due to excellent nutraceutical properties. Carroll (1990) successfully incorporated stabilized rice bran in bakery products up to 20%.

Among the various problems emerging out as a consequence of population growth, the problem of food particularly of good quality protein is reaching to its critical level affecting adversely the health and vitality of the people. As chapatti is the main and major source of protein and energy for the people of Asian sub continent (7.70% of the total dietary requirement). About 124 kg wheat flour in the form of chapatti is consumed per capita per annum. However it can not supply adequate amount of nutrients. Moreover, the wheat protein is also deficient in Lysine, methionine and threonine amino acids necessary for maintenance and growth (Saunders, 1990). Thus it seems feasible to supplement the wheat flour with other sources like PRB which are rich in these different amino acids.

Rice bran, a by-product of rice milling industry is a good source of protein, energy, minerals and vitamins (B-complex and tocopherols) etc. (Dale, 2000). Its nutrients density i.e. amino acids and fatty acids profiles (75% unsaturated fatty acids) are superior to cereal grains (Farrell, 1999). The proteins and fats are relatively of high biological value (Saunders, 1990
and Sayre et al., 1988) Thus the present study was initiated to supplement wheat flour with different levels of PRB (0-30%) on w/w basis to improve the nutritional value of chapati.

4.12 Nutritional Evaluation of Processed Rice Bran Through Broiler Chicks Feeding Trials

4.12.1 Experiment-1 Replacement of maize with processed rice bran

This study was conducted to evaluate the nutritive value of PRB by comparing the performance of broiler chicks for weight gain, feed consumed, feed conversion ratio (FCR), dressing percentage, liver, gizzard and pancreas weights by incorporating raw RRB and various processed rice brans (PRB-I, PRB-II and PRB-III) replacing maize and 100% lysine with PRB (w/w) from a conventional control broiler ration, with the objective that PRB can be used as cereal substitute for improved nutritional quality because poultry feed comprised of up to 65% cereal grains. Different performance parameters pertaining to present study are presented in Table 26.

The data subjected to statistical analysis, using analysis of variance techniques revealed significant (P≤0.01) difference in weight gain, feed consumed, FCR, dressing percentage and pancreas weight while liver and gizzard weight showed non-significance difference among various treatments (Table 26).

The significance of mean differences for various parameters of rations are presented in Table 27. The weight gain of chicks fed on various rations indicated a significant difference in weight gain of ration containing PRB-II (T₂) (2410 g) as compared to all other rations (T₁, T₃, T₄ and T₅). The lowest weight gain was found in ration containing raw PRB (T₂). The chicks fed on PRB (extruded, acidic and alkaline) gained 138, 260 and 223g more weight than that of control in which only maize was added in the ration. The growth rate was depressed (1710 g) i.e. 20.47% in ration
Table 26. Mean squares for performance of broiler chicks fed on diets containing maize replaced with processed rice bran.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Avg. wt. gain/ chick (g)</th>
<th>Avg. liver wt. (g)</th>
<th>Avg. gizzard wt. (g)</th>
<th>Avg. pancreas wt. (g)</th>
<th>FCR</th>
<th>Dressing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>10</td>
<td>49207.217**</td>
<td>96269.972**</td>
<td>87.036**</td>
<td>0.410**</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td></td>
<td>4717.381</td>
<td>116.565**</td>
<td>3.927</td>
<td>6.338</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>26630.091</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = P≤0.01  
NS = Non significant
Table 27. Performance of broiler chicks fed on diets containing maize replaced with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight gain/chick (g)</th>
<th>Feed consumed/chick (g)</th>
<th>Feed Conversion Ratio</th>
<th>Dressing %</th>
<th>Liver weight (g)</th>
<th>Gizzard weight (g)</th>
<th>Pancreas weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>2150d</td>
<td>5380b</td>
<td>2.50c</td>
<td>61.98a</td>
<td>49.49a</td>
<td>33.49a</td>
<td>5.12b</td>
</tr>
<tr>
<td>T₂</td>
<td>1710e</td>
<td>4990c</td>
<td>2.92d</td>
<td>57.01b</td>
<td>48.54a</td>
<td>35.80a</td>
<td>6.46a</td>
</tr>
<tr>
<td>T₃</td>
<td>2288c</td>
<td>5490a</td>
<td>2.40b</td>
<td>63.30a</td>
<td>48.94a</td>
<td>34.05a</td>
<td>5.18b</td>
</tr>
<tr>
<td>T₄</td>
<td>2410a</td>
<td>5350b</td>
<td>2.22a</td>
<td>64.85a</td>
<td>51.37a</td>
<td>35.01a</td>
<td>5.21b</td>
</tr>
<tr>
<td>T₅</td>
<td>2373b</td>
<td>5510a</td>
<td>2.32b</td>
<td>62.45a</td>
<td>48.92a</td>
<td>35.76a</td>
<td>5.32b</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control (maize basis)
T₂ = RRB (Raw)
T₃ = PRB-I (extruded)
T₄ = PRB-II (1% acetic acid)
T₅ = PRB-III (1% Ca(OH)₂)
containing 20% raw rice bran (T₂) when compared with control (T₁). It was further observed that all the rations were significantly (P≤ 0.05) different with one another for gaining weight. These results indicated that partial replacement of maize by processed rice brans increased the weight gain of chicks. The chicks fed on ration T₂ showed a significant decrease in weight gain than the chicks fed on control (T₁).

The highest feed consumption was recorded in chicks fed on T₅ (5510 g) and it was significantly different from feed consumption of chicks fed on ration T₁, T₂ and T₄ while significantly the lowest feed consumption ratio was observed (4990 g) in chicks fed on T₂ i.e. raw rice bran. The chicks fed on T₁ and T₄, T₃ and T₅ rations showed statistically non-significant differences with one another. The ration T₂ (RRB) was less consumed (7.25%) by the chicks than control (T₁) ration while the utilization of rations containing PRB was comparable with the control (T₁) ration.

The results regarding feed conversion ratio (FCR) of chicks fed on rations T₁, T₂, T₃, T₄ and T₅ are shown in Table 27. The chicks fed on T₂ (2.92) and T₄ (2.22) showed significantly higher FCR than the chicks fed on rations containing treatments (T₁, T₃ and T₅) while rations of T₃ and T₅ showed statistically non-significant difference. The FCR of rations containing processed rice brans ranged from 2.22 to 2.40 against 2.50 in case of control ration. The chicks fed on ration T₂ showed the highest FCR (2.22) while ration T₄ exhibited the lowest FCR (2.92) when compared with T₁ (control ration). Chicks fed on T₄ ration attained 11.2% higher FCR. The data indicated that the processing of rice bran improved its nutritive value. Thus it indicated that incorporation of PRB in rations enhanced its FCR.

After slaughtering the chicks, the dressing percentage was calculated by removing feathers, skin, half head and vesra i.e. toe with feet, lungs and gastro intestinal tract. Organs (liver, heart and gizzard)
were properly cleaned and weighed along with dressed carcass. When the means of the data were subjected to statistical analysis, the results revealed that dressing percentage was found to be significantly higher in chicks fed on T₁, T₃, T₄ and T₅ than chicks fed on T₂ (Raw rice bran) ration. The dressing in record rations of T₁, T₃, T₄ and T₅ rations were found to be non significantly different. The data indicated that the dressing percentage of control and all the rations containing PRB (T₁, T₃, T₄ and T₅) was statistically identical. The highest dressing percentage was obtained in T₄ ration and minimum in T₂ ration.

The comparison of mean values for liver and gizzard weight of various rations (T₁-T₅) are presented in Table 27. The results revealed a statistically non-significant differences among all the rations.

The mean weights of pancreas have been presented in Table 27. The checks fed on ration T₂ showed significantly the highest pancreas weight. This indicated that anti-nutritive factors present in rice bran affected this organ and its secretions. Its weight increased with the presence of trypsin inhibitors in rice bran. The rations containing PRB, the trypsin inhibitors were inactivated by various treatments (extruded, acid and alkali). The pancreas weight of chicks fed on T₁, T₃, T₄ and T₅ rations were non-significantly different with one another but showed significant differences for chicks fed on raw rice bran (T₂).

So PRB can replace 20% maize, which is more expensive than rice bran and always in short supply as its production is low and the demand high.

A significant decrease in growth rate (live weight gain), feed-consumption and utilization (FCR) was noted in chicks fed on ration T₂ containing 20% raw rice bran (RRB) as compared to control (T₁) ration. A decrease in dressing percentage also occurred in ration of control (RRB). It might be due to decline in body weight gain (growth depression), poor feed
consumption and conversion efficiency of chicks fed on RRB ration. Where as Liver and Gizzard weights were not affected by the incorporation of RRB in T₂, no Gizzard erosion was observed. Their weights were recorded to be normal as per control in RRB and PRB rations (T₂ to T₅). These were found to be non-significantly different. The decrease in above parameters might be due to the presence of toxic or anti nutritive factors present in raw rice brans (RRB).

The results of the present study are in concordance with the findings of Saunders (1990), Barber et al. (1978), Shaheen et al. (1975), Warren and Farrell (1990), Takemasa and Hijikuru (1991) and Majid (1997). The improvement in growth of chicks fed on rations containing processed rice brans (PRB I, PRB II, PRB III) supported the presence of some toxic factors in raw rice bran (RRB). These toxic or anti nutritional factors were inactivated or eradicated by moist heat treatment (extrusion). The nutritive value of moist extruded rice bran (T₂) was further improved by the processing with acetic acid and calcium hydroxide. The results are fully in conformity with the findings of another researcher like Tsai, (1976) who reported that mixing of moist rice before autoclaving at 120 °C improved the feed efficiency of chicks. The reason for improvement in growth response and feed efficiency of chicks fed on processed rice brans, might be due to removal or lowering of anti-tryptic activity, breakdown of calcium-magnesium-phytate complex, inactivation of lipase and haemagglutinin activity etc. These views are further supported by the investigation of other scientists that growth of chicks was enhanced by extrusion cooking and addition of calcium hydroxide in the ration (Sayre et al. 1987 and 1988; Tsai, 1976). Other toxic factors i.e. haemagglutinin as reported by Ory et al. (1981) and Tsuda (1979), lipase by Desikacher (1974) and phytates reported by Tangendjaja and Lowary (1985) and Thompson and Weber, (1981), were reduced or removed.
The results of the present study are also identical to Kratzer et al. (1974) who found that the processing of rice bran with acetic acid (1%) and extruded (steam) denatured the toxic factors which improved the growth and feed efficiency of chicks. The pancreatic weight showed hypertrophy in RRB ration but for PRB rations, it remained normal. These findings are in agreement with the work of Kratzer et al. (1976) who concluded that pancreas weight of chicks was normal by feeding extruded rice bran as compared to raw rice bran. This might be due to the trypsin inhibitors present in RRB.

It may be concluded from the results that acetic acid treatment combined with extrusion cooking of rice bran improved the nutritive value of rice bran and also minimized the toxic factors.

4.12.2 Feed Cost of Broiler Chicks (maize replacement)

In the present study, the data on feed cost/chicks, feed cost/kg live weight gain and feed cost/kg dressed meat were tabulated and subjected to statistical analysis, using analysis of variance techniques (Table 28). The results of analysis of variance showed significant (P<0.05) differences among all these studied parameters.

The feed cost/chicks (Rs.) of various rations (T₁ - T₅) has been given in Table 29. The feed cost of ration T₁ (control) in which only 30% maize was added showed significant (P ≤ 0.05) differences with all other rations of treatments (T₂, T₃, T₄ and T₅). The feed cost of ration T₂ in which 20% maize was replaced with raw rice bran showed no significant differences with feed cost of control (T₁) ration. The feed cost of rations of treatments T₃, T₄ and T₅ in which 20% of different PRB has been incorporated by replacing 20% maize indicated statistically non-significant differences with one another and it was significantly lower than the price of control ration. Low cost of raw rice bran ration is due to no physical and chemical treatment to this bran (T₂) and its price is much lower than maize.
Table 28. Mean squares for feed cost of broiler chicks fed on diets containing maize replaced with processed rice bran

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Feed cost/chick (Rs.)</th>
<th>Feed cost/kg live wt. gain (Rs.)</th>
<th>Feed cost/kg dressed meat (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>83.769**</td>
<td>29.588**</td>
<td>92.525**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>2.168</td>
<td>0.801</td>
<td>1.995</td>
</tr>
</tbody>
</table>

** = P ≤ 0.01
Table 29. Means for feed cost of broiler chicks fed on diets containing maize replaced with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed cost/chick (Rs.)</th>
<th>Feed cost/kg live wt. gain (Rs.)</th>
<th>Feed cost/kg dressed chicks (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>56.49a</td>
<td>26.27a</td>
<td>42.47b</td>
</tr>
<tr>
<td>T₂</td>
<td>46.16b</td>
<td>26.99a</td>
<td>47.35a</td>
</tr>
<tr>
<td>T₃</td>
<td>52.16c</td>
<td>22.80b</td>
<td>35.97c</td>
</tr>
<tr>
<td>T₄</td>
<td>53.50c</td>
<td>22.20b</td>
<td>34.29d</td>
</tr>
<tr>
<td>T₅</td>
<td>52.90c</td>
<td>22.29b</td>
<td>35.74c</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control  
T₂ = RRB (raw)  
T₃ = PRB-I (extruded)  
T₄ = PRB-II (1% acetic acid)  
T₅ = PRB-III (1% Ca(OH)₂)
The results regarding feed cost/kg live weight gain in chicks have been presented in Table 29. Statistical analysis showed non-significant differences among feed cost/kg live weight gain rations of treatments T₁ and T₂ and between rations of T₃, T₄ and T₅ treatments. The substitution of PRB, decreased the feed cost/kg live weight gain by Rs.13.21, 15.49 and 15.15% respectively as compared to control (T₁) ration.

The feed cost / kg dressed meat was found to be higher for T₂ ration and it decreased for T₃, T₄ and T₅ rations respectively. The data were subjected to statistical analysis (Table 29) and significant differences were found in feed cost / kg dressed meat of various rations. The feed cost/kg dressed meat of T₃ and T₅ rations was found to be non-significantly different while T₁ and T₂ ration showed statistically significant (P ≤ 0.05) differences. The feed cost/kg dressed meat was found to be significantly the lowest in chicks fed on T₄ as compared to all other rations. It was further observed that PRB–III with 20% maize found to be the best on economic basis i.e. feed cost/kg dressed meat reduced Rs. 8.18 as per control.

It was obscured that the feed cost (Rs.) per kg live weight gain was reduced by the supplementation of PRB with maize (20%) in the rations (T₃, T₄ and T₅). So these rations containing processed rice bran (PRB) were more economical in terms of cost factor parameters when moist acetic acid followed by extrusion processing of rice bran was compared with control (T₁). But for RRB, feed cost per kg live weight gain was higher than control in which 20% maize was added in the ration. After this treatment (T₄) the cost reduced by Rs.4.07 (15.49%) as compared to control but for RRB ration, the cost increased by Rs.0.72 (2.74%) than that of control ration. The feed cost per kg dressed meat decreased by 15.30, 19.26 and 15.85% for rations containing rice bran having extrusion, acid and alkali processing, respectively than the control ration. The feed cost/kg
dressed meat increased by 11.49% when raw rice bran was substituted with 20% maize. The increase in feed cost might be due to depressed growth and low feed efficiency of RRB. These results are fully in agreement with the findings of Chauhan and Sharma (1996), Mahbub et al. (1989).

Tiemoko (1992) who concluded that rations having treated defatted rice bran in their formulation were economical (reduced feed cost per kg weight) than those containing maize. It was concluded that replacement of maize (20%) with PRB was economical but moist acetic acid plus extrusion was found to be the most effective and significantly improved the feed cost by 15.49 and 19.26% for weight gain and dressed meat per kg which may be exploited in ration formulation of chicks.

4.12.3 Experiment-II. Replacement of wheat with processed rice bran

An other similar experiment was conducted to study the performance of broiler chicks for weight gain, feed consumption, dressing percentage and organs weight (Liver, Gizzard and Pancreas), by replacing wheat with raw and PRB in the formulation of various rations.

The analysis of variance indicated statistically significant ($P \leq 0.01$) differences in weight gain and pancreas weight. The feed consumed, feed conversion ration (FCR) and dressing percentage also showed statistically significant ($P \leq 0.05$) differences. The Liver and Gizzard weights showed non-significant differences among various treatments (Table 30). This indicated that substitution of PRB with wheat showed significant affect on the performance of birds.

The weight gain showed significant differences among chicks fed on all rations (Table 31). The chicks fed on rations of $T_4$ and $T_5$ treatments showed non-significant difference with one another for weight gain. The weight gain per bird decreased 14.14% for $T_2$ and increased 8.9, 27.9 and 23.8% respectively when chick were fed on $T_3$, $T_4$ and $T_5$ rations containing
Table 30. Mean squares for performance of broiler chicks fed on diets containing wheat replaced with processed rice bran.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Weight gain/chick (g)</th>
<th>Feed consumed/chick (g)</th>
<th>Feed Conversion Ratio</th>
<th>Gizzard weight (g)</th>
<th>Liver weight (g)</th>
<th>Pancreas weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>151,375.26**</td>
<td>133,946.841*</td>
<td>0.044*</td>
<td>2.826 NS</td>
<td>0.568</td>
<td>0.285**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>7468.688</td>
<td>23863.878</td>
<td>0.013</td>
<td>4.004</td>
<td></td>
<td>0.047</td>
</tr>
</tbody>
</table>

** = P≤0.01  * = P≤0.05  NS = Non significant
Table 31. Means for performance of broiler chicks fed on diets containing wheat replaced with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight gain/chick (g)</th>
<th>Feed consumed/chick (g)</th>
<th>Feed Conversion Ratio</th>
<th>Dressing %</th>
<th>Liver weight (g)</th>
<th>Gizzard weight (g)</th>
<th>Pancreas weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>2009b</td>
<td>4360c</td>
<td>2.17b</td>
<td>61.15a</td>
<td>48.42a</td>
<td>33.86a</td>
<td>5.06b</td>
</tr>
<tr>
<td>T_2</td>
<td>1725c</td>
<td>4297c</td>
<td>2.49a</td>
<td>55.75b</td>
<td>47.92a</td>
<td>33.18a</td>
<td>6.15a</td>
</tr>
<tr>
<td>T_3</td>
<td>2188b</td>
<td>4537bc</td>
<td>2.07b</td>
<td>63.12a</td>
<td>48.97a</td>
<td>34.02a</td>
<td>5.20b</td>
</tr>
<tr>
<td>T_4</td>
<td>2570a</td>
<td>4787ab</td>
<td>1.86c</td>
<td>64.99a</td>
<td>50.32a</td>
<td>34.25a</td>
<td>5.16b</td>
</tr>
<tr>
<td>T_5</td>
<td>2487a</td>
<td>4929a</td>
<td>1.98c0</td>
<td>62.39a</td>
<td>49.58a</td>
<td>34.52a</td>
<td>5.23b</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T_1 = Control
T_2 = RRB (raw)
T_3 = PRB-I (extruded)
T_4 = PRB-II (1% acetic acid)
T_5 = PRB-III (1% Ca(OH)_2)
different processed as compared to control (T₁) ration in which 15% wheat was incorporated in the ration. It was further observed that significantly higher weight gain was observed in chicks fed on T₄ ration, which was 27.9% more than control (T₁) ration.

The mean values for feed consumption have been described in Table 31. The Chicks fed on ration T₂ consumed 63g less feed as compared to chick fed on control (T₁) ration. However the chicks on feeds T₃, T₄ and T₅ ration consumed 177, 427 and 569 g more feed than chicks on the control ration. It was obvious that the chicks on ration T₄ and T₅ utilized more feed. Statistically non-significant differences were observed in feed consumption among T₁, T₂ and T₃ rations while T₃ and T₄, T₄ and T₅ were also non-significantly different with one another with respect to feed consumption.

The feed consumption ratio (FCR) of rations containing various PRB (T₃, T₄ and T₅) were 2.07, 1.86 and 1.98 respectively. The chicks fed on ration T₄ showed the lower FCR (1.86) while chicks fed on T₂ (raw rice bran) indicated the highest FCR (2.49) when compared with the birds fed on control ration T₁. The data on FCR when subjected to statistical analysis, the results for FCR values showed a statistically non-significant difference among the means of T₁, T₂ and T₃ as well as T₄ and T₅ rations. Thus by replacing 15% wheat with PRB improved the FCR.

The dressing percentage have been given in Table 31. The dressing percentage of rations fed on chicks T₁, T₂, T₃, T₄ and T₅ rations was found to be 61.15, 55.75, 63.12, 64.99 and 62.99% respectively. The dressing percentage of chicks fed on ration containing raw rice bran (T₂) was found to be significantly lowest as compared to all other rations. The dressing percentage of chicks fed on T₁, T₃, T₄ and T₅ rations was found to be non significantly different.
The mean values pertaining to Liver and Gizzard weights for various experimental rations showed statistically non-significant differences among T₁, T₂, T₃, T₄ and T₅ ration (Table 31).

The differences for pancreas weight of various experimental rations in Table 31 showed that the weight for chicks fed on T₂ was the highest (5.64g). The results indicated that pancreatic weights of chicks fed rations T₁, T₃, T₄ and T₅ were statistically non-significant with one another while pancreas weight of chicks fed on T₂ ration showed significantly the highest as compared to all other treatments. By the supplementation of raw rice bran (15%) the pancreas weight increased by 11.5% as compared to control. The other treatments (T₂, T₃, T₄ and T₅) were statistically comparable with one another and control for processed weight.

The ration (T₂) containing 15% raw rice bran (RRB) replaced by wheat, significantly depressed growth rate (live weight gain) and feed consumption caused poor feed efficiency ratio (FCR) as compared to control (T₁) ration. The dressing percentage also decreased in RRB (T₂). It might be due to low weight gain and poor feed consumption and utilization but there was no effect of raw or processed rice brans on Liver and Gizzard weights. These were non-significantly different among all experimental rations. The decrease in above parameters might be due to the presence of growth depressant in raw rice bran.

These anti-nutritive factors might be lipases, trypsin inhibitors, haemagglutinin-lactin and phytates etc. present in raw rice bran as reported by Barber et al. (1978), Tsai (1976), Takemasa and Hijikuro (1991), Thompson and Weber (1981), Ory et al. (1981), Desikacher (1977), and Saunders (1990). Majid (1997) also expressed that body weight gain, feed intake and utilization (FCR) of chicks fed various treated defatted rice brans were significantly higher than the chicks fed on untreated defatted rice bran. So the treatments improved the nutritive
value of defatted rice bran. But in the present study the supplementation of 15% PRB (PRB-I, PRB-II and PRB-III) to wheat enhanced the chicks growth even with ration having 15% wheat (control). Maximum improvement in the chicks growth and feed efficiency was observed in PRB II (T₄) which indicated that acetic acid treated moist rice bran with extrusion process was a potent tool to get rid of the growth depressing factors.

The results are also in concordance with Eggum et al. (1984) who found that wet heat treatment destroyed the antitryptic activity. Kratzer (1976), Sabir et al. (1980) and Saunders (1990) reported that lipase activity was checked by heating the rice bran. The results in the present study are confirmed by Sayre et al. (1988) who found that supplementation of rice bran with calcium source improved the weight gain but the present study is also in line with Deolanker and Singh (1979) who reported that by autoclaving, trypsin inhibitor was decreased but not completely eradicated. This may be due to the reason that they did not apply moist extrusion cooking, as in present study extrusion plus acetic acid or alkaline processing of rice bran was carried out. It is obvious from the present study that moist extrusion or chemical treatment followed by extrusion decreased and inactivated the toxic factors from rice bran. Kratzer et al. (1974) indicated that processed rice bran (acetic acid plus extension cooking), denatured the toxic proteins which improved the growth and feed efficiency of birds. They further found that pancreas weight of chicks was normal by feeding extruder cooked rice bran as compared to raw rice bran. The raw rice bran showed hypertrophy of pancreas weight. It might be due to the toxic factors present in raw rice bran. The present study suggests that processed rice bran can be substituted with wheat to improve the nutritional status of the diet which have been reflected by the performance of PRB in different parameter of chicks.
4.12.4 Feed Cost of Broiler Chicks (wheat replacement)

An other similar study was conducted to evaluate the feed cost (economical) of broiler chicks by substituting wheat with 15% raw and processed rice brans. Various feed cost attributes of chicks pertaining to present investigation are summarized in Table 32.

The analysis of variance, indicated statistically significant (P≤0.05) difference among feed cost/chicks, feed cost/kg live weight gain and feed cost/kg dressed meat (Table 32).

The mean values for feed cost/chick, feed cost/kg live weight gain and feed cost/kg dressed meat are presented in Table 33.

The feed cost/chick of various rations (T₁ - T₅) were compared (Table 33) which indicated significant (P ≤ 0.05) differences among chicks fed on T₁, T₂ and T₃ rations while T₁, T₄ and T₅ rations showed statistically non-significant differences. The results indicated that feed cost reduced to 17.13, 8.69, 1.40 and 2.17% respectively by incorporation of raw rice bran and PRB to various rations (T₂, T₃, T₄ and T₅) as compared to control (T₁) ration. The highest feed cost was given by the control ration, T₁ (Rs. 53.41) and the lowest for T₂ (Rs. 44.26) in which wheat was replaced with raw rice bran. PRB rations are relatively more costly than T₂ due to processing of rice bran.

Feed cost depends upon price of raw material, processing and its consumption etc. The cost of feed varies but usually it is low when production of raw material is on its peak. Feed cost/chick may vary due to its formulation too. The results pertaining to differences in feed cost/kg live weight gain of rations (T₁ – T₅) are presented in Table 33. The highest feed cost/kg live weight gain was recorded in chicks fed on control (T₁) ration (Rs. 26.59) and the lowest in chicks fed on T₄ (Rs. 20.49). With the substitution of PRB in wheat, the feed cost reduced by Rs. 4.30, 6.10 and 5.58, respectively when chicks fed on T₃, T₄ and T₅ rations, respectively.
Table 32. Mean squares for feed cost of broiler chicks fed on diets containing wheat replaced with processed rice bran

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Feed cost/chick (Rs.)</th>
<th>Feed cost/kg wt. gain (Rs.)</th>
<th>Feed cost/kg dressed chicks (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>4</td>
<td>15.415**</td>
<td>15.168**</td>
<td>30.268**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>3.517</td>
<td>0.173</td>
<td>1.807</td>
</tr>
</tbody>
</table>

** = P≤0.01
Table 33. Means for feed cost of broiler chicks fed on diets containing wheat replaced with processed rice bran

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed cost/chick (Rs.)</th>
<th>Feed cost/kg wt. gain (Rs.)</th>
<th>Feed cost/kg dressed meat (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>53.41a</td>
<td>26.59a</td>
<td>43.42b</td>
</tr>
<tr>
<td>T₂</td>
<td>44.26b</td>
<td>25.66a</td>
<td>46.10a</td>
</tr>
<tr>
<td>T₃</td>
<td>48.77c</td>
<td>22.29b</td>
<td>35.34c</td>
</tr>
<tr>
<td>T₄</td>
<td>52.66a</td>
<td>20.49c</td>
<td>31.53d</td>
</tr>
<tr>
<td>T₅</td>
<td>52.25a</td>
<td>21.01bc</td>
<td>33.71c</td>
</tr>
</tbody>
</table>

Mean values sharing similar letters in a column are not significantly different

T₁ = Control
T₂ = RRB (raw)
T₃ = PRB-I (extruded)
T₄ = PRB-II (1% acetic acid)
T₅ = PRB-III (1% Ca(OH)₂)
The results further revealed that $T_3$ showed statistically significant differences for feed cost/kg live weight with $T_1$, $T_2$, $T_4$ and $T_5$ rations while $T_4$, $T_5$ and $T_1$, $T_2$ rations were found to have non-significant differences with respect to feed cost/kg live weight.

Similar pattern was observed for feed cost per kg dressed meat (Table 33). The feed cost/kg dressed meat of chicks fed on $T_2$ were ranked at the top (Rs. 46.10) while chicks fed on $T_4$ ration ranked at the lowest (Rs. 31.53). The substitution of PRB (RRB, PRB-I, PRB-II and PRB-III) in various rations ($T_2$, $T_3$, $T_4$ and $T_5$), increased the feed cost by Rs. 4.44% for $T_2$ ration and it decreased by Rs. 19.94, 28.57 and 23.63%, respectively when chicks fed to $T_3$, $T_4$ and $T_5$ rations as compared to the control ($T_1$) ration. From this investigation, it is obvious that substitution of 15% PRB with wheat decreased the feed cost per kg dressed meat than the control ration. It may be inferred that the processed rice bran can be an excellent substitute of wheat up to 15% for high quality yield of chicks.

The results of the present study clearly indicated that minimum feed cost (Rs) per kilogram live weight gain was observed in chicks fed on ration ($T_4$) which contained 15% processed rice bran which was chemically and physically treated with moist acetic acid plus extrusion against chicks fed on control ration in which only 15% wheat was incorporated (without incorporation of raw and processed rice brans). Similar pattern was observed for feed cost per kg dressed meat. The PRB rations ($T_3$ to $T_5$) surpassed the ration $T_1$ (control) and $T_2$ (RRB) and presented promisingly lower feed cost per kg dressed meat.

These findings are in concordance with the findings of Malik (1976) and Sabir (1980) who concluded that rations having rice bran in their formulations were economical than those containing maize. The present study is also confirmed by Mahbub et al. (1989), Tiemoko (1992) and Chauhan and Sharma (1996) who reported that rations having treated
defatted rice brans in their formulations were economical. This study again suggested that chicken must be fed on rations which contain processed rice bran especially T₄ for getting more yield and good quality of chicken meal.
Chapter-V

SUMMARY

Rice bran is a by-product of rice milling industry, abundantly and cheaply available in rice producing countries. Its full nutrients potential cannot be utilized due to presence of indigenous toxic factors such as lipase, lipo-oxygenase, trypsin inhibitors, haemagglutinin-lectin and phytates etc. These antinutritional factors affect the digestibility and quality of nutrients. These factors must be inactivated / eliminated to achieve a highly digestible nutrients profile of rice bran. In the present study a process has been developed to inactivate the toxic factors present in rice bran in a single operation.

Freshly obtained raw rice bran was thoroughly mixed with 20% simple water or 20% solution of 1% acetic acid or calcium hydroxide and allowed to equilibrate for one hour. Then the treated rice brans were cooked by passing through an extruder cooker maintained at a temperature of 130±2°C for 10-15 seconds. The treated and extruded (processed) rice bran were dried to less than 10% moisture. The nutritive value of processed rice brans was compared with the raw rice bran through chemical, biological and statistical methods.

The result indicated that rice bran contained crude protein 13%, crude fat 12.93%, crude fiber 7.65%, ash 10.34% and nitrogen free extract 56.08%. Processing (treatment + extrusion cooking) showed no negative effect on proximate (protein, fat etc) composition of rice brans. Available phosphorous increased by 89.13%.Cell wall decreased (ADF and NDF) and cell contents increased on chemical treatments. Bulk density and storability (90 days) increased of processed rice. The color remained off-white and structure improved from fluffy powder to crumbles.
As regarding anti-nutritional factors, the FFA in different treated rice brans varied from 0.08 to 3.20, 0.07 to 0.20, 0.01 to 0.08 and 0.04 to 0.15% during 90 days storage. The lipase was inactivated by moist, acidic or alkaline processing of rice brans. The peroxidase activity decreased in all the processed rice brans at 0, 5, 10 and 15 seconds interval. The diminishing trend from 47 to 12, 40 to 2 and 43 to 8 in moist, acidic and alkaline processed rice brans respectively was recorded during 15 seconds. Acetic acid treated rice bran (PRB-II) showed maximum decrease than that of calcium hydroxide treated rice bran (PRB-III).

Haemagglutinin-lectin activity was 23.92 of raw rice bran reduced to 0.96/mg on processing of rice bran. The maximum reduction was observed in chemically processed rice bran. Unprocessed bran had 8018 trypsin inhibitor unit per gram. On processing the rice bran, the activity was reduced to zero, indicating a 100% inactivation of trypsin inhibitor. Phytate in raw rice bran was 4.25% which was reduced to 54.12, 89.88 and 77.65% by simple water, acidic and alkaline solution respectively in moist and processed rice brans. The effect of processing on microbial load of rice bran indicated that the total viable count and mould count per gram in rice bran dropped to negligible on processing. The best treatment having less anti nutritional factors (acetic acid treated) was selected for further supplementation in wheat flour for preparation of bread, cookies and chapatti.

The breads were prepared by incorporation of processed rice bran (PRB-II) from 0, 5, 10, 15, 20, 25, 30% replacing wheat flour on w/w basis. The results showed that the crude protein, crude fat, crude fiber and ash contents increased whereas nitrogen free extract decreased by the addition of PRB. Sensory evaluation of breads was performed by panel of judges for external and internal characteristics. The sensory scores improved by adding processed rice bran up to 15%, then the scores
decreased. The total of external and internal characteristics scores was calculated. The processed rice bran supplemented breads exhibited 77.66, 78.73 and 80.30 score for 5, 10 and 15% processed rice bran supplemented breads.

Cookies were prepared by incorporation of processed rice bran (0-30) to wheat flour. The chemical analysis of cookies showed that protein, fat, fiber and ash contents of cookies increased and NFE contents decreased in PRB-II supplemented cookies. Sensory parameters of cookies i.e. color, crispness, taste, texture acceptability and total scores increased by the incorporation of 5, 10 and 15% PRB to wheat flour on w/w basis. The scores decreased when 20, 25 and 30% PRB was added to wheat flour.

Chapati is a staple food and provides about 90% of daily protein and energy requirements but wheat is deficient in lysine. Rice bran is rich source of lysine and oil, which can supplement wheat flour. The chapaties were prepared from whole wheat flour (WWF) (100% extraction) and supplemented with 0, 5, 10, 15, 20, 25 and 30% PRB-II, replacing WWF on w/w basis.

The results of chemical analysis of chapaties showed that crude protein increased from 11.23 to 12.83%, fat from 1.98 to 9.47% crude fiber 2.11 to 3.79% and ash from 1.4 to 5.21% by the allocation of PRB in WWF. The NFE decreased from 83.27 to 68.70%.

The results of sensory evaluation of various chapaties by panel of judges showed that scores for color, flavour, taste, texture, feel to touch, foldability and breakability improved, i.e. the color 7.41 to 8.07, flavour 6.25 to 8.80, taste 7.81 to 8.80, texture 6.78 to 6.91, feel to touch 7.15 to 7.27, foldability 7.33 to 7.35 and sum of all the scores 49.54 to 53.90 for chapaties supplemented to 15% PRB to wheat flour. Beyond this level i.e. 20 to 30% PRB supplementation the scores decreased progressively. The
chapaities that scored less points than WWF chapaities were considered to be rejected by judges during sensory evaluation test.

Biological assay of chapaities showed that weight gain of rats fed WWF was 63 g which increased by 7, 10 and 19 g when fed 5, 10 and 15% PRB chapaities, respectively. True digestibility of all chapaities showed non-significant differences in chapaities. The biological value of PRB supplemented chapaities also increased by 6, 11 and 13% which contained 5, 10 and 15% PRB. The net protein utilization (NPU) of chapaities containing PRB increased substantially. The results showed that supplementation of WWF with PRB up to 15% on w/w basis increased nutrients density, acceptability and nutritive value of chapaities.

The processed rice bran were used in broiler chicks feeding trials, replacing 20% (w/w) maize with processed rice bran from a control (standard) diet. The results showed that the chicks fed with processed rice bran gained more weight than control diet containing 20% maize or unprocessed diet. The chicks gained 29% weight when rice bran was incorporated in the diet with 13% better feed conversion ratio than chicks fed on control. Cost of feed per kilogram weight gain was reduced by 20% by adding processed rice bran.

The broiler chicks feeding experiment in which 15% wheat was replaced in the diet by processed rice bran, the results indicated that increase in weight gain as compared to the control were 15.30, 19.26 and 15.85% for 5, 10 and 15% processed rice bran, respectively. The feed cost per kg dressed meat decreased by Rs 8.08, 28.8 and 22.9% by use of 5, 10 and 15% PRB, respectively.

This study indicated that rice bran should be treated with acid or alkali with subsequent extrusion cooking to inactivate/ minimize the antinutritional factors. The processed rice bran can be used up to 15% for the preparation of bread, cookies and chapaities. The nutritional value of
chapatis containing processed rice bran exhibited higher nutritional attributes such as gain in weight, PER, NPU and biological value as compared to whole wheat flour chapaties. The diets containing processed rice bran also enhanced more weight of chick with feed conversion ratio.

Thus processed rice bran can be used for the preparation of high nutritive breads, cookies and chapaties in order to overcome malnutrition problems prevailing among masses in Pakistan. This will also help to utilize the rice bran and it will also release pressure on wheat to some extent.
CONCLUSION

It can be concluded from the findings of the present study that

1. Non-significant differences existed in various constitutes like protein, fat, carbohydrate and minerals in between raw and processed rice brans.

2. The total starch and phosphorus did not change due to processing of rice but the increase in the available phosphorus was observed due to hydrolysis of phytates in processed rice brans.

3. The acid detergent fibers (ADF) and neutral detergent fibers (NDF) decreased increasing the values of hemicellulose on processing of rice bran. The bulk density increased on processing. Color and keeping quality was also improved on processing of rice bran.

4. Undesirable and antinutritional factors like lipase, peroxidase, haemagglutinin-lectin, trypsin inhibitors and phytates were inactivated and minimized on processing of rice brans. Microbial load i.e. bacteria mold and insects pests were also killed giving toxcine free sterile product. Shelf life of processed rice bran increased more than 90 days.

5. the supplementation of wheat flour with processed rice bran upto 15% for the preparation of bread, biscuits and chapaties enhanced chemical constituents like protein, fat, fiber and ash contents. The nutritional assay revealed significant improvement in protein efficiency ratio, net protein utilization, biological value by the incorporation of 15% processed rice bran in wheat flour.

6. The results suggested that rice bran should be treated with calcium hydroxide with subsequent extrusion for inactivation/elimination of anti nutritional factors. The use of
processed rice bran upto 15% can be used for the higher preparation of chapaties, breads and biscuits. This will help to combat malnutrition existing among various groups in Pakistan.

RECOMMENDATIONS

- Anti nutritional factors like lipase, lipo-oxidase, trypsin inhibitors, haemagglutinin-lectin and phytates can be inactivated through moist, acidic or alkaline processing of rice bran to utilize their nutrient potential.
- The processed rice bran can be supplemented up to 15% in wheat flour for the preparation of bread, biscuits and chapaties.
- The processed rice bran can be used in the feeds of broiler chicks replacing 20% (w/w) maize or 15% (w/w) wheat.
LITERATURE CITED


Lowry PD and Gill CO. 1994. Temperature and water activity minimum for
growth of spoilage moulds from yeast. J. Appl. Bacteriol. 56:
193-199.

Luh BS, Barber S and Benedito-de BC. 1991. Rice Bran: Chemistry and
Technology. In: Rice Production and Utilization, Vol. II (Luh BS.,


Mahbub AS, Rehman MA, and Raza A. 1989. Use of rice polish as partial
replacement in growing chicks. Bangladesh J. Anim. Sci. 18(1-
2):99-104

Adviser 18:39-41.

Majid A. 1997. Effect of various treatments of rice bran on the performance

Malik MY and Chughtai MID. 1979. Chemical composition and nutritive value

Malik MY. 1976. Effect of feeding different levels of rice bran (solvent
extracted) on the growth rate and feed utilization of broiler

Marcel Dekker, Inc., New York.

Cleveland, Ohio 441052, USA.

2nd ed. CRC Press, Boca Raton, FL.


extrusion cooking for extraction of edible oil. J. Food Sci. 50:361-368.


155


**APPENDIX-I**

**COMPOSITION OF VITAMIN PREMIX**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>200 mg</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>100 mg</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>250 mg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>250 mg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5 g</td>
</tr>
<tr>
<td>Pentothenic Acid</td>
<td>500 mg</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>4 g</td>
</tr>
</tbody>
</table>
APPENDIX-II

COMPOSITION OF MINERAL PREMIX

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium citrate 4H₂O</td>
<td>308.2 g</td>
</tr>
<tr>
<td>Ca (H₂PO₄)₂.H₂O</td>
<td>112.8 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>218.7 g</td>
</tr>
<tr>
<td>KCl</td>
<td>124.7 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>77.0 g</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>68.5 g</td>
</tr>
<tr>
<td>3MgCO₃.Mg(OH)₂.3H₂O</td>
<td>35.1 g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>38.3 g</td>
</tr>
</tbody>
</table>

**Trace Minerals**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNH₄ Citrate</td>
<td>91.41 g</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>5.98 g</td>
</tr>
<tr>
<td>NaF</td>
<td>0.76 g</td>
</tr>
<tr>
<td>MnSO₄.2H₂O</td>
<td>1.07 g</td>
</tr>
<tr>
<td>KAl(SO₄)₂.12H₂O</td>
<td>0.54 g</td>
</tr>
<tr>
<td>KI</td>
<td>0.24 g</td>
</tr>
</tbody>
</table>

\[\text{Total:} \ 16.7 \text{ g}\]

\[\text{100.00 g} \ \ \ \ \ \ \ \ \ \text{1000.00 g}\]