

DEVELOPMENT AND EVALUATION OF STABILIZED RICE BRAN INCORPORATED BAKED PRODUCTS

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B.Tech. (Food Science & Technology)

**MASTER OF SCIENCE
(FOOD TECHNOLOGY)**



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**DEVELOPMENT AND EVALUATION OF
STABILIZED RICE BRAN INCORPORATED
BAKED PRODUCTS**

BY

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B.Tech. (Food Science & Technology)

**THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR
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FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
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2016

DECLARATION

I, **KRISHNEGOWDA K S**, hereby declare that the thesis entitled **“DEVELOPMENT AND EVALUATION OF STABILIZED RICE BRAN INCORPORATED BAKED PRODUCTS.”**Submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Master of Science in Food Technology** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place:

(KRISHNEGOWDA K S)

Date:

I.D. NO. FST/2014-015

CERTIFICATE

Mr. KRISHNEGOWDA K S. has satisfactorily prosecuted the course of research and that thesis entitled “**DEVELOPMENT AND EVALUATION OF STABILIZED RICE BRAN INCORPORATED BAKED PRODUCTS**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any university.

Date:

Chairperson
(Dr. K. UMA MAHESWARI)

CERTIFICATE

This is to certify that the thesis entitled “**DEVELOPMENT AND EVALUATION OF STABILIZED RICE BRAN INCORPORATED BAKED PRODUCTS.**” submitted in partial fulfillment of the requirements for the degree of “**MASTER OF SCIENCE IN FOOD TECHNOLOGY**” of the **Professor Jayashankar Telangana State Agricultural University, Hyderabad** is a record of the bonafide original research work carried out by **Mr. KRISHNEGOWDA K S** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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9	IX	Estimation of ash	
10	X	Estimation of carbohydrate	
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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	At the rate
<	:	Less than sign
>	:	More than sign
±	:	Plus-Minus symbol
µg	:	Microgram
µl	:	Microliter
ANOVA	:	Analysis of Variance
AOAC	:	Association of Analytical Chemists
AOAC	:	Association of Official Analytical Chemists
ARI	:	Agriculture Research Institute
ASRB	:	Acid Stabilized Rice Bran
b*	:	Brightness
C	:	Control sample
C H Sc.,	:	College of Home science
cm	:	Centimeter
CD	:	Critical Difference
CFU/ml	:	Colony Forming Units per milliliter
⁰ C	:	Degree Celsius
CDRB	:	Commercially Defatted Rice Bran
DH	:	Dry Heating
DPPH	:	1,1-diphenyl-2-picryl-hydrazyl
DRB	:	Defatted Rice Bran
DOM	:	Degree of milling
e.g.	:	for example, for instance
EDTA	:	Ethylene tetra diamine
ESC	:	Escape
<i>et al.</i>	:	and other people
etc.	:	and so on; and other people/things

EXT	:	Extrusion
EERB	:	Enzymatic Extract Rice Bran
EV	:	Ethanol Vapor
FAO	:	Food and Agriculture Organization
FDDH	:	Freeze Drying Followed by Dry heating
FFA	:	Free Fatty Acid
Fig	:	Figure
FTIR	:	Fourier transform infrared spectrometry
g	:	Gram
g/l	:	Gram per liter
h	:	Hour
H ₂ O	:	Water
H ₂ SO ₄	:	Sulphuric acid
HCL	:	Hydrochloric acid
HDL	:	High density lipo protein
HDPE	:	High density poly ethylene
HDRD	:	Heat stabilized defatted rice bran
HSRB	:	Heat stabilized rice bran
i.e	:	Means
kg	:	Kilogram
l	:	Liter
L*	:	Lightness
LDRB	:	Laboratory defatted rice bran
mg	:	Milligram
MHz	:	Mega hertz
MH	:	Microwave heating
min	:	Minute(s)
ml	:	Milliliter(s)
MT	:	Million Ton
Na ₂ CO ₃	:	Sodium carbonate
NADH	:	Nicotinamide Adenine Dinucleotide

NaOH	:	Sodium hydroxide
No.	:	Number
PAR	:	Parboiled rice bran
PEF	:	Pulsed electric field
PGRC	:	Post graduate and research center
pH		Log of H ⁺ ion concentration
PJTSAU	:	Professor Jayashankar Telangana State Agricultural University
RBA	:	Rose bengal agar media
SD	:	Standard deviation
SF	:	Spread factor
Sec	:	Second(s)
Se	:	Selenium
SD	:	Standard deviation
STC	:	Steam cooking
SWRB		Stabilized whole rice bran
T1	:	Baked products prepared by incorporated 10% of whole rice bran
T2	:	Baked products prepared by incorporated 10% of stabilized whole rice bran
T3	:	Baked products prepared by incorporated 20% of whole rice bran
T4	:	Baked products prepared by incorporated 20% of stabilized whole rice bran
T5	:	Baked products prepared by incorporated 30% of whole rice bran
T6	:	Baked products prepared by incorporated 30% of stabilized whole rice bran
TBARS	:	Thiobarbituric acid reactive substance
TBC	:	Total Bacterial Count
TCA	:	Trichloro acetic acid

TMC : Total Mould Count
Un-RB : Unstabilized rice bran
viz. : Namely
WRB : Whole rice bran
wt Weight

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ABSTRACT

Milling of paddy to obtain edible rice grain yields two major by-products of economic and nutritional importance, namely, paddy husk and rice bran. Paddy husk has no food value but has several industrial uses. Rice bran can serve as a human food supplement and as a valuable source of edible oil. Both the bran and oil from rice bran have a range of bioactive phytochemicals with potential for reducing the risk of chronic degenerative diseases. There is a need to utilize the full potential of the available rice bran in the country, both as a source of healthy edible oil and as a food supplement for promoting human nutrition and health.

There baked products (biscuit, cookies and cakes) were developed by using wheat flour and rice bran at three different combinations (100:0, 90:10, 80:20 & 70:30) with two types of rice bran (whole rice bran and stabilized whole rice bran). Totally six variations were formulated viz. WRB 10%, SWRB 10%, WRB 20%, SWRB 20%, WRB 30% and SWRB 30% for the study.

Statistically significant difference was observed in moisture, ash, fiber, fat, carbohydrate and iron content of the whole rice bran and stabilized whole rice bran. The moisture content was significantly higher ($P < 0.05$) for the whole rice bran (10.77 ± 0.30) compared to the stabilized whole rice bran (7.11 ± 0.17). Statistically significant difference in the protein and calcium content was observed between the WRB and SWRB.

Statistically significant difference was observed in bulk density but not in water absorption capacity between the whole rice bran and stabilized whole rice bran samples. L^* value was significantly higher ($P < 0.05$) for the whole rice bran (62.77 ± 0.44) compared to the stabilized whole rice bran (58.73 ± 0.78). The a^* and b^* values were significantly lower ($P < 0.05$) for the whole rice bran (1.81 ± 0.24 and 18.22 ± 0.65) compared to the stabilized whole rice bran (2.48 ± 0.15 and 20.33 ± 0.87) sample.

Sensory evaluation was done for the control sample and for all the six variations to know the most acceptable baked products from each of the variations. They were evaluated for color, texture, taste, flavor, and overall acceptability. Mean sensory scores revealed that biscuit and cakes were best accepted at 10% level of WRB and SWRB incorporation. Cookies were best accepted at 10% incorporation of WRB and 20% in SWRB respectively.

Physical properties of baked products were affected significantly with the increase in the level of incorporation of bran. Addition of increasing amount of rice bran from 0 to 30 % decreased diameter and spread ratio and increased thickness of biscuits and cookies. Addition of increasing amount of rice bran from 0 to 30 % decreased height, length, width, volume and density of cakes.

The result of chemical and nutritional quality characteristics of baked products (biscuits, cookies and cakes) revealed that statistically significant difference ($P < 0.05$) was observed between the control sample and rice bran incorporated baked products. The protein, fat, carbohydrate, ash and crude fibre, were higher in both WRB and SWRB incorporated baked products compared to control.

There was a decreasing trend of sensory scores for color, texture, taste, flavor, and overall acceptability for control, raw rice bran and stabilized rice bran incorporated baked products, during shelf life study and significant difference was found between the treatments and duration for all the sensory characteristics.

The total bacteria and fungi counts were observed in the products at the end of the storage period *i.e.* in biscuit and cookies at 30th day. In cake bacteria and fungi were observed on 3rd to 6th day of storage period. The total bacteria and fungi count was significantly higher in whole rice bran incorporated baked products compared to control and stabilized whole rice bran incorporated baked products.

The findings of the present study strongly support that the stabilized rice bran can be successfully incorporated in baked products as it enhances the nutritional quality, functional properties and shelf life of the baked products. Rice bran, a "little known" food is highly nutritious and delivers a powerhouse of health supporting nutrients which is either thrown away or used for low-level animal feed. In the view of popularity of rice bran and its therapeutic use, it was proposed to process the rice bran and to develop food products to enhance the nutrient contents.

Chapter I

INTRODUCTION

Rice is the staple food of 65% of population in India. The major rice growing countries are China, India, Indonesia, Bangladesh, Thailand, Burma, Vietnam, Japan and Philippines. Rice is the largest consumed calorie source among the food grains, with a per capita availability of 73.8 kg it meets 31% of the total calorie requirement of the population. India is the second largest producer of rice in the world next to China. In India paddy occupies the first place both in area and production. Apart from rice milling, processing of rice bran for oil extraction is also an important agro processing activity for value addition, income and employment generation (Quereshi *et al.*, 2000).

Milling of paddy to obtain edible rice grain yields two major by-products of economic and nutritional importance, namely, paddy husk and rice bran. Paddy husk has no food value but has several industrial uses. Rice bran is an inexpensive, underutilized milling by-product of rough rice. Rice bran is the cuticle existing between the rice and the husk of the paddy and consists of embryo and endosperm of the seeds of *Oryza sativa*, family *Graminae*. Rice bran, on the other hand, can serve as a human food supplement and as a valuable source of edible oil.

Rice bran - both full fat and defatted is a rich source of nutrients and can serve as a source of nutrient supplement. Both the bran and oil from rice bran have a range of bioactive phytochemicals with potential for reducing the risk of chronic degenerative diseases. There is a need to utilize the full potential of the available rice bran in the country, both as a source of healthy edible oil and as a food supplement for promoting our population's nutrition and health. The yield of husk bran and milled rice from 100 kg paddy are 22.8 kg and 73.0 kg, respectively. The yield of bran depends upon the degree of milling of the brown rice; it may vary from 5 to 10%. In India, polishing is restricted to 5% by Government regulation (Narasinga Rao, 1988).

Rice bran constitutes 8% of the weight of the whole grain and contains most of the nutrients (65%), such as vitamins, minerals, oils, trace elements, antioxidants,

phytosterols and phytochemicals. It contains 12-22% oil, 11-17% protein, 6-14% fiber, 10-15% moisture and 8-17% ash. It is rich in vitamins including vitamin E, thiamin, niacin and minerals like aluminium, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc (Mccaskill & Zhang, 1999).

The main drawback of rice bran is a fast oxidation reaction due to the high content of unsaturated fatty acid in rice bran oil. This is primarily due to the presence of endogenous enzyme lipase which causes the pro-oxidative mechanisms of oxidation leading to hydrolytic rancidity on the oil content that hydrolyze the ester bonds of triacylglycerol, releasing fatty acids and glycerol and forming of hyper oxides (Yin and Wen, 2011). Within one hour of separating the bran from the grain during milling, the material turns rancid liberating toxic free fatty acids. These shortcomings have now been overcome by destroying the lipolytic activity using an advanced stabilizing technology the resulting material thus obtained is called "stabilized" rice bran which has a good taste, readily soluble with a longer shelf life of one year.

Rice bran is used for the enrichment of foods, due to its high dietary fiber content. Since the middle of the 1970s, the role of dietary fiber in health and nutrition has stimulated a wide range of research activities which caught public attention. Accumulating evidence favors the view that increased intake of dietary fiber can have beneficial effects against diseases, such as cardiovascular diseases, gastrointestinal diseases, decreasing blood cholesterol, diverticulosis, diabetes and colon cancer. In view of the therapeutic potential of dietary fiber, more fiber incorporated food products are being developed. Addition of dietary fiber to a wide range of products will contribute to the development of value-added foods or functional foods that currently are in high demand (Hu *et al.*, 2009).

Rice bran also plays an important role in decreasing cholesterol and controlling of blood glucose level. Stabilized rice bran (SRB), is a powerful source of vitamins, nutrients, proteins and fiber. The soluble and insoluble fibers are necessary for optimum digestion, blood sugar regulation, lowering cholesterol and prevention of diabetes and heart diseases. The, stabilized rice bran contains an approximate insoluble versus soluble fiber ratio of 5 to 1, which exhibits a high digestive tolerance that occurs along the whole digestive tract with no excessive fermentation in the large intestine. Processed rice bran contains astounding quality of synbiotics, tocopherols, oryzanol, polyphenols, sitosterol, phytosterols and is packed with full of omega-3 and omega-6 fatty acids. Healthy

complex carbohydrates found in processed rice bran have "low glycemic index" which means they do not cause spikes in blood glucose (Sayre *et al.*, 2007).

Rice bran, a "little known" food is highly nutritious and delivers a powerhouse of health supporting nutrients which is either thrown away or used for low-level animal feed. In the view of popularity of rice bran and its therapeutic use, it was proposed to process the rice bran and to develop food products to enhance the nutrient contents.

Rice bran is finding enormous applications in food industries for increasing the nutritional quality of processed foods. Rice bran being high in dietary fiber and in view of its therapeutic potential, its addition can contribute to the development of value-added foods or functional foods that currently are in high demand. Supplementation of rice bran has been successfully carried in various foods like bread, cakes, noodles, pasta and ice creams without significantly affecting the functional and textural properties (Saunders, 1985).

Therefore, in the present investigation, an attempt was made to develop and standardize method for stabilization of rice bran, to develop baked products (biscuits, cookies and cakes) by incorporating rice bran and to test the shelf life of the developed products. In view of the above, the present investigation was undertaken with the following objectives:

1. To standardize the process for stabilization of rice bran.
2. To formulate and standardize stabilized rice bran blended baked products.
3. To assess physicochemical and nutritional quality characteristics of stabilized rice bran and rice bran incorporated backed products.
4. To evaluate the sensory quality characteristics of rice bran incorporated backed products.
5. To conduct shelf life studies for the best selected formulation.

Chapter II

REVIEW OF LITERATURE

The study on development and evaluation of stabilized rice bran incorporated baked products was conducted in the Post Graduate & Research Centre, Department of Foods & Nutrition, C H Sc., PJTSAU, Rajendranagar, Hyderabad. Available relevant literature to present study has been reviewed under the following headings.

- 2.1. Production of rice bran
- 2.2. Processing of rice bran
- 2.3. Nutrient composition of rice bran
- 2.4. Rice bran incorporated products
- 2.5. Shelf life study of the developed products

Bakery industry is the one of the largest food industries in India with an annual turnover of Rs. 300000 crores (Ahmad and Ahmed, 2014). Biscuit along with bread forms major baked food accounting to over 30% and 50% respectively of total bakery products produced in the country. The industries have been established in organized and unorganized sectors contributing equal share. India's bakery market is the third largest market in Asia pacific, only after Japan and Australia (Sheereen, 2013).

Enrichment of food products with functional components has been commonly used in order to enhance their pro-health properties. Due to their widespread consumption, cereal food products, which in developed communities provide more than 50% of the total energy intake, are considered to be the best vehicles for functional supplements (Akhtaret *al.*, 2011). Precisely because of its simplicity and its wide consumption, the bread is suitable to be enriched and fortified with ingredients that can bring benefits to the consumer in terms of health (Danzaet *al.*, 2014). Currently, there are some successful trials concerning the improvement of the nutraceutical potential of bakery product through enrichment (Dzikiet *al.*, 2014).

Nutritional and functional properties of rice bran are well suited for baked products like cookies, muffins, bread, crackers, pastries and pancakes (Barber *et al.*, 1981).

2.1. Production of rice bran

Hisamitsu takai and Barredo (1981) carried out series of milling tests with long- and bold-grained rice, IR 36 and they stated that process of milling can be divided into husking and polishing; husking was rapid, while polishing was relatively slow. Prolonged continuous-milling increased the degree of polishing slightly, but caused a drastic increase in breakage. The intermittent-milling permits an over milling without an increase in breakage. They finally concluded that no reduction in the percentage of broken rice which was achieved by 2-stage milling (Husking by a rubber roll huller and then polishing by a mill).

Kunlunet *al.* (2009) investigated the relationships between physical properties, degree of milling (DOM) and loss of selenium (Se) in 10 brown rice cultivars during milling process. Most of the physical properties varied very significantly with different cultivars except true density and porosity. They also significantly affected DOM and the loss of Se during the milling process. Among the physical properties investigated, grain length, aspect ratio and porosity played a very significant role in controlling DOM and the loss of Se. A linear relationship was further observed between DOM and the loss of Se.

Kshirod (2011) reported that the extent to which bran is removed during milling (or whitening) is called the degree of milling (DOM) of rice. The DOM affects rice quality in many ways. As the bran layer has a different composition, the DOM affects the chemical composition of rice. Fat gets smeared on the grain surface during partial milling; hence DOM affects flow and packing properties of rice as well as storage stability. Many micronutrients are concentrated in the bran layer; hence DOM of rice affects its nutritive value. DOM affects cooking too as the bran layer offers some resistance to cooking.

Pradeepet *al.* (2013) studied the effect of processing such as steaming, germination and parboiling on nutraceutical and *in vitro* bioactive properties of rice bran from three different rice varieties namely Jyothi (pigmented), IR64 and Sonamasuri (non-pigmented). Within the varieties envisaged, pigmented Jyothi variety contained higher levels of vitamin E, soluble, bound and total polyphenol, flavonoids, free radical scavenging activity and total antioxidant activity. Direct steam exposure of bran resulted in an increase in, ether extractives and oryzanol, as well as retention of all the vitamin E components. Parboiling as well as germination of paddy resulted in an increase, in the

content of ether extractives and oryzanol, whereas other bioactive properties decreased compared to native. Hence it may be concluded that bioactive components and antioxidant properties were significantly higher in Jyothi bran compared to the other two paddy brans, and processing leads to changes in bioactive properties with maximum retention of bioactive components in the steamed bran.

Flavia *et al.* (2014) evaluated the proximate composition, colour, total flavonoids, anthocyanins and proanthocyanidins content, as well as the total phenolics and antioxidant activity of IAC-600 black rice cultivar and MPB-10 red rice. Black rice showed a thicker bran layer than red rice. Ash content of red and black rice was around 80% and 65% respectively. 4% DOM reduced 47% of the fat content in red rice, while in order to reduce similar fat content in black rice, a 7% DOM was necessary. The total free phenolics were around 6 and 7-fold higher than bound phenolics for black and red rice, respectively.

Nesse (2015) investigated the composition and hydrolytic deterioration behavior of rice bran fractions which were obtained individually from different rice whitening mills. Additionally, stabilization of these bran fractions individually with middle infrared radiation and its effects on the contents of tocopherols and α -oryzanol were investigated. Free fatty acid (FFA) content of the crude and stabilized bran fractions that were obtained from the last whitening and polishing steps was higher either in the beginning or in the end of the storage compared to the others obtained in the first steps of whitening. Stabilization at 700 infrared (medium-wave) power for 7.0 min provided 90 days of shelf life without a notable change in FFA content of rice bran fraction which was obtained from the first whitening step.

2.2. Processing of rice bran

Tao *et al.* (1993) studied the microwave heating as an effective method for the inactivation of lipase that is responsible for rice bran degradation and instability. Rice bran stabilized by microwave heating at 2450 MHz for 3 min, found to be stable up to four weeks in storage. Free fatty acid content of microwave stabilized long grain rice bran increased from 4.0 to 4.9 % and from 4.6 to 6.25 % in medium grain rice bran, even when stored under unfavorable storage conditions ($33^{\circ}\pm 2^{\circ}\text{C}$, $75\pm 5\%$ relative humidity). In contrast, untreated bran FFA ranged from 4.0 to 68.3 % and 4.6 to 56.8 % in medium grain bran respectively.

Qinger *et al.* (1998) studied the stabilization process of rice bran immediately after milling by added-moisture heating and dry heating. During experimental storage, the result showed that the added-moisture heating was very efficient for the lipase inactivation. The free fatty acid values were less than 10% for the samples processed at 110°C and 120°C for 3 or 5 mm. after 40-days storage. While the dry-heated rice bran could only be stored for about 15 days with the free fatty acid value around 15%, since the lipase activity was gradually recovered with the absorption of ambient moisture.

Ramezanzadeh *et al.* (2000) reported the effect of microwave heat, packaging methods and storage temperatures on proximate and fatty acid compositions of rice bran during 16 weeks of storage was examined. Freshly milled raw rice bran, adjusted to 21 % moisture content and microwave heated for 3 min. Raw and microwave heated bran were packed in zipper-top bags and vacuum-sealed bags and stored at 4-5°C and 25°C for 16 weeks. The moisture content decreased significantly from an initial 8.4 to 6.4% in microwave-heated samples regardless of packaging methods and storage temperatures. Protein, fat, linoleic and linolenic acid contents did not change significantly in all raw and microwave-heated samples during 16 weeks of storage. The microwave-heated rice bran packed in zipper-top bags can be stored at 4-5°C for up to 16 weeks without adverse effect on proximate and fatty acid composition quality.

Rao *et al.* (2003) investigated and the usage of ohmic heating was used to stabilize rice bran and to improve rice bran oil extraction yield and compared with microwave heating and a control (no heating). Results showed that ohmic heating is an effective method for rice bran stabilization with moisture addition. Free fatty acid concentration increased more slowly than the control for raw bran samples subjected to ohmic heating with no corresponding temperature rise, indicating that electricity has a non-thermal effect on lipase activity. Ohmic heating increased the total percent of lipids extracted from rice bran to a maximum of 92%, while 53% of total lipids were extracted from the control samples. Lowering the frequency of alternating current significantly increased the amount of oil extracted, probably due to electroporation. Ohmic heating was successfully applied to rice bran despite its high oil content.

Marco *et al.* (2005) evaluated the stability of the rice bran treated by four stabilization processes was evaluated during four months of storage. They are non-stabilized polished rice bran, toasted polished rice bran, cheese whey-stabilized polished rice bran, non-

stabilized parboiled rice bran, toasted parboiled rice bran, and cheese–whey-stabilized parboiled rice bran. The alcohol-soluble and water-soluble acidity of rice bran stored at 48°C, every week, for four months was evaluated. The process of cheese–whey addition increased the content of calcium in bran. Parboiled rice bran (PAR) was the most stable sample during the storage, suggesting that the parboiling gives greater stabilization to the bran.

Hettiarachchy (2009) studied on yeast fermentation of rice bran extracts. Heat-stabilized defatted rice bran (HDRB) was fermented with yeast to afford a food product having superior prebiotic for probiotic properties. Fermentation of HDRB with yeast yields a prebiotic composition that can promote the growth or activity of beneficial intestinal bacteria (probiotic) when consumed by an animal or human. Inclusion of legumes during fermentation can synergistically enhance the bioactivities of HDRB. Heat-stabilized defatted rice bran can be a source of growth medium for yeast/mold/microorganisms.

Rosniyana *et al.* (2009) evaluated rice bran at 4 % and 8 % milling degree and stabilized by either autoclaving or parboiling process. The rice bran was autoclaved with commercial retort at 120°C for 20 min. For the production of parboiled rice bran, the harvested paddy was soaked for 2 h, steamed for 20 min then dried and milled. The free fatty acid levels for both parboiled and autoclaved rice bran were below the 10 % permissible level for 4 months and 6 months respectively for the product packed in oriented polypropylene/ polypropylene packs, either vacuumed or without, and stored in ambient temperature room condition. The storage of rice bran by polypropylene packs, as control packaging material, led to rapid production of free fatty acids. These findings indicate that rice bran can be stored without risk of deterioration for a substantial time prior being used for the production of many health-related food products.

Abdel (2013) reported the effect of some thermal processing (Microwave, Parboiled, Roasted and Hot air) on stability of rice bran during storage in polyethylene bags at room temperature for 8 weeks. The results showed that, stabilization treatment improved oil extraction yield. The moisture content of roasted rice bran was lower than those of stabilized and unstabilized rice bran. In addition, the moisture content of rice bran samples decreased with increasing storage period. Thermal processing decreased the free fatty acids of rice bran after 8 weeks compared with unstabilized rice

bran. Thermal processing showed an increase in palmitic and stearic acids, while linoleic and linolenic acids were decreased. Saturated fatty acids were increased after 8 weeks of storage period. Parboiled rice bran had comparatively higher levels of protein, fat and ash contents than unstabilized (Un-RB) and other stabilized rice bran. Thus the microwave stabilization of rice bran has advantages over the other stabilization method.

Jianet *al.* (2014) studied the feasibility of Pulsed Electric Field (PEF) on low moisture food materials. Based on a monolayer of brown rice grains the results showed that the lipase activity could be significantly inactivated by PEF. Among the PEF parameters, the voltage was the most important to the inactivating efficiency, followed by frequency and pulse width; while the time was less dominant. The interactions between voltage and pulse width and between frequency and pulse width also contributed to the lipase inactivation significantly.

Sunget *al.* (2014) evaluated the various heat treatments, such as dry-heating (DH), freeze-drying followed by dry-heating (FDDH), microwave heating (MH), autoclaving (AC), and ethanol vapor (EV) treatment were applied to rice bran and their effects on the storage stability at room temperature were evaluated. The free fatty acid (FFA) content of untreated rice bran gradually increased from 2.14 to 19.81% during 24 weeks, whereas those of the treated rice brans were not or marginally changed. The FFA content in dry-heating samples was greater than that in freeze drying samples, indicating the positive effect of freeze-drying prior to dry-heating. Among the treatments, autoclaving was most effective in retarding the FFA formation. Amounts of other bio-functional components such as phytosterols and policosanols are increased by these treatments.

Edenilseet *al.* (2015) investigated the effects of an acetic and propionic acid mixture on the proximal composition, colorimetric profile, gross energy and lipid oxidation products in rice bran during storage. Whole rice bran treated with organic acids and stored for 120 days exhibited lower quality. However, no major proximal composition alterations were observed during storage or related to organic acid use. Further more, organic acids yielded the highest gross energy values, lower lipid acidity increases, less primary and secondary lipid oxidation product formation, and maintenance of the yellow color (value b*) after storage for 120 days. This study shows that applying an acetic and propionic acid mixture conserves the bran well.

2.3. Nutrient composition of rice bran

Amissahet *al.* (2003) conducted study on the nutrient composition of 16 bran samples from new rice varieties. Parameters measured were moisture, fat, crude fiber, protein, ash and mineral content. Carbohydrate and energy levels were calculated from the appropriate data obtained. Results showed significant differences in fat, crude fiber and ash contents of samples. With the exception of GRUG 7, ITA 334 and ITA 402, significant differences existed in moisture content. Significant variations also existed in protein content with the exception of ITA 304 and ITA 334. The bran from the different rice varieties had appreciably high levels of K, P and Ca. Energy levels were within 300 Kcal/ 100g range except that of GRUG 7 and BETA which were lower. Ca: P ratios were quite appreciable making the potential use of the samples for high feed preparation.

Sudarathet *al.* (2005) studied on the chemical composition of full fat and defatted rice bran for protein, fat, moisture content, fibre and ash, using AOAC (1995). The results revealed that full fat rice bran contains 8.5% of moisture, 12.6% of protein, 21.13% of fat, 5.59% of crude fibre, 8.97% of ash and 43.12% of carbohydrate whereas the defatted rice bran contains 12.43% of moisture, 13.89% of protein, 1.92% of fat, 6.03% of crude fibre, 10.13% of ash and 55.6% of carbohydrate.

Azizahet *al.* (2007) reported the chemical composition of local stabilized rice bran. The four rice-bran milling fractions, after stabilization by microwave heating on site at the rice mill, were analyzed for their chemical composition. The content of all fractions tested (in g/100 g) consist of 8.7–18.9% of fat, 8.8–15.2% of protein, 8.5–12.6% of moisture, 4.2–7.7% of ash, 22.2–44.8% of total carbohydrates (by difference) and 18.3–30.5% of total dietary fibre. It is encouraging to note that total phenolic compounds of all fractions were detected at 257–488 mg ferulic acid equivalent per 100 kg, while carotenoid contents were found to be in the range of 58.7–216 mg/100g. The amino acid composition varied within wide limits with proline, histidine and threonine as the amino acids. Higher concentrations of amino acids found (in decreasing order) were arginine, glutamic acid, aspartic acid and serine. Phosphorus and potassium were among the major mineral constituents of rice bran, with values up to 1633 mg/100g. The first rice bran fraction was found to be highest in energy, fat and minerals and could be a very good source of dietary fiber and other nutrients.

Elisaet *al.* (2009) evaluated the nutraceutical composition of a water-soluble enzymatic extract from rice bran (EERB) and its antioxidant and hypocholesterolemic activities. The EERB contains broad functional components in water soluble form such as sterols, tocopherols, tocotrienols, *c*-oryzanol and peptides. The antioxidant behavior of EERB specifically against lipid oxidation was measured in an emulsion prepared with fish oil containing fatty acid. The emulsion showed a stable antioxidant activity, specifically preserving the lipid from peroxidation phenomena and inhibiting the generation of thiobarbituric acid-reactive substances (TBARS) and lipid peroxides. Moreover, the hypocholesterolemic activity was evaluated in male Wistar rats through two different hypercholesterolemic diet models. Reduction in total cholesterol levels and an increase in HDL-cholesterol were found in both models. EERB is a new product from rice bran that can control lipid oxidation in emulsions and induce a physiological hypocholesterolemic effect. These findings may facilitate the development and use of new products derived from rice within the functional food field.

Rao *et al.* (2010) studied the antioxidant and antiproliferative potential of rice bran extracted from an important Indian rice variety, Njavara and compared the same with two commercially available basmati rice varieties. Rice bran methanolic extract from Njavara showed the highest antioxidant and cell cytotoxic properties compared to the other three rice varieties. The value for scavenging DPPH and nitric oxide were in the range of 30.85-87.72 $\mu\text{g/ml}$ and 52.25-107.18 $\mu\text{g/ml}$ respectively. Thus it is concluded that crude methanolic extract from Njavara rice bran contains significantly high polyphenolic compounds with superior antioxidant activity as evidenced by scavenging of free radicals including DPPH. It is conceivable that the Njavara rice variety could be exploited as one of the potential sources of plant-based pharmaceutical products.

Fariaet *al.* (2012) subjected the rice bran samples to two stabilization methods to inactivate enzymatic deterioration. Changes in nutritional value in terms of, concerning chemical composition, minerals and fatty acid content, were evaluated to supplement existing data and promote the utilization of rice bran in the human diet. The following homemade heat treatments were applied: roasting on a conventional stove or heating in a microwave oven. Results showed that different heating methods affected sample composition, since the levels of some nutrients of treated samples showed significant changes compared to corresponding raw samples. The rice bran treated on a conventional

stove produced products with lower moisture (5.14 ± 0.10 g/100g) and nutrients such as sodium 11.8%, palmitic acid 9.9% and stearic acid 8.1%. The microwave oven procedure resulted in better nutrient preservation, with slightly higher moisture content (6.28 ± 0.10 g/100 g), and appears to be a practical and rapid tool for home heat stabilization of rice bran.

Abdel-Galeel *et al.* (2012) investigated the effect of milling degree on nutritive value of rice bran that stabilized by extrusion process. The hulled rice was milled for three durations (30, 60 and 90 sec.) to obtain three fractions of rice bran (1st, 2nd and 3rd). The chemical composition of moisture, protein, lipids, ash and dietary fiber were evaluated. Results indicated that all fractions of rice bran contain high values of lipids, ash and dietary fiber. The rice bran is very rich in phosphorus (1635mg) and potassium (1453mg). In addition, it contains suitable amounts of all essential and nonessential amino acids. Thus it is concluded that the 1st fraction of rice bran contains higher values of protein (13.4%), ash (8.1%), phenolic compounds and dietary fiber (29.6%) as well as it has more amounts of essential amino acids and all elements in comparing with those of other rice bran fractions.

Khalid *et al.* (2015) reported that the Rice bran is a rich source of vitamins, minerals, essential fatty acids, dietary fiber and other sterols. There is a wide spread scientific agreement on various health benefits associated with consumption of dietary fiber. Consumer attitude towards health foods is promising and the scope of functional foods is growing in the world markets; rice bran is finding increased applications in food, nutraceutical and pharmaceutical industries. However, potential applications of rice bran in food industry are limited by its instability owing to rancidity caused by exposure of oil to lipases during milling. Various methods of stabilization have been carried out, paving way for supplementation of rice bran in numerous food preparations.

2.4. Rice bran incorporated products

Shaheen *et al.* (2005) studied the suitability of processed and treated rice bran for the supplementation of bread. The treated and extruded rice bran was supplemented at 5, 10, 15, 20, 25 and 30% in wheat flour for the production of bread. The bread was analyzed for different physicochemical parameters and subjected to sensory evaluation. The results showed an increase in crude protein from 11.87 to 12.94%, crude fat from 3.64 to 8.63%, crude fiber 0.62 to 2.15% and ash 1.52 to 4.18%. The sensory evaluation showed significant differences in the scorers for volume, color of crust, symmetry of form and character of crust. The breads supplemented with 15% incorporation got high sensory scores. It can be concluded from the results that up to 15% processed rice bran can be successfully incorporated in the bread to improve the sensory and nutritional attributes.

Quilez *et al.* (2008) formulated bread, where wheat flour was replaced by 3%, 6% and 9% of rice bran (RB) previously exposed to two types of heat treatment: extrusion (EXT) and steam cooking (STC). The aim was to investigate how RB affected the properties of wheat-flour dough and partially baked wheat bread, as a final product. The results of the study indicate that neither of the RB treatments had a negative influence on the rheological characteristics of the dough, but that its fermentation capacity and gas retention improved, especially in case of EXT-RB, which was exposed to more intensive heat treatment. In the final bread product, there was also a positive increase in the specific total volume for the EXT-RB bread, and a decrease for the STC-RB bread. Nevertheless, sensory analysis showed that the score for the RB enriched bread decreased when RB content increased. They concluded that low concentrations of RB exposed to a more intensive heat stabilization process improves several rheological properties of the dough and the physical properties of the partially wheat bread.

Saeed *et al.* (2009) reported on the impact of adding 2-20% rice bran to wheat flour on the rheological behavior of the dough using Farinograph, Consistograph and Alveograph. The changes in physico-chemical properties of dough were insignificant even after including 20% of the bran. The wheat flour and rice bran mixtures were used to prepare cookies and flat bread (*chapati*) and the sensory evaluation was carried out for the products. The sensory scores of the product, such as texture and taste, decreased with increased substitution with the rice bran. It was concluded that cookies and *chapati*, up to 10% rice bran replacement were rated acceptable.

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

Bagheri and Seyedein (2011), conducted study to increase the fiber content of bread, with 5, 10, 15 and 20% incorporation of rice bran. Baking and rheological tests on breads showed that an increase in the amount of rice bran leads to a rise in the extension coefficient as well as water absorption, but it decreases flour resistance to extension. The result of sensory evaluation revealed that the sample containing 10% rice bran had the best quality.

Sairamet *al.* (2011) studied the effect of physico-chemical characteristics, antioxidant potential of defatted rice bran (Laboratory-LDRB and Commercial-CDRB) and its utilization in preparation of bread. The effect of incorporation of CDRB at varying levels (5, 10 and 15%) on the quality characteristics of bread including physical, rheological and sensory attributes were evaluated and the dietary fiber content and antioxidant activity were determined. On the basis of physical characteristics, breads with 5% and 10% CDRB were found to be acceptable. The dietary fiber content and total antioxidant activity of bread increased with increasing levels of CDRB, which also improved the shelf life.

Younaset *al.* (2011) developed the cookies from wheat flour with supplementation of rice bran at 5, 10, 15 and 20%. The rice bran was stabilized with acid and dry heat treatment before supplementation. Chemical analysis of the cookies revealed that there was no significant difference in chemical and physical properties of cookies supplemented with acid stabilized rice bran (ASRB) and heat stabilized rice bran (HSRB). The moisture, crude protein, fat and mineral contents were significantly increased with the increment of rice bran. Average width, thickness and spread factor of cookies also increased with the increase in percentage of rice bran. Sensory evaluation of cookies showed that scores for

color of cookies decreased significantly with increase in level of rice bran and sensory scores were significantly higher in the cookies prepared with HSRB. Hence it is concluded from the results that supplementation of HSRB at 10% is more suitable for production of rice bran supplemented cookies.

Mishra and Chandra (2012) studied the possibility of fortifying the soya flour and rice bran to formulate the functional biscuit which have the ability to improve the quality of food products due to various functional properties. Supplementation of wheat flour with soya and rice bran was tried at 10, 15, 20 and 25% level each. Prepared biscuit was subjected to physical, sensory and nutritional analysis to evaluate the suitability of biscuit for consumption. The width of biscuit decreases from 44 to 36.2 with increasing in the level of substitution of composite flour of rice bran and soya. Similar trend was shown by spread ratio. Biscuit thickness increased from 9.2 to 10.6, with increasing level of substitution. nine-point hedonic score system was used for sensory evaluation of prepared biscuit which is generally decreases with increasing the level of substitution. Thus, it has been concluded that supplementation of soya flour and rice bran at 15% level each, would improve the nutritional quality without adversely affecting the sensory parameters.

Marina *et al.* (2012) reported the viability of using microwave-roasted rice bran as an ingredient in high-fiber cereal bars to obtain a product with good acceptability. The influence of the rice flakes, corn flakes, and roasted rice bran levels on the physical and chemical characteristics of the cereal bars was studied. The overall acceptability of three selected formulations was also evaluated. An increase in the roasted rice bran level in the formulation reduced the force of rupture and water activity, resulted in intermediate density, and caused darkening of the bars. The contents of lipid and total dietary fiber were higher in the formulation with the highest rice bran content, which was therefore classified as functional food. Cereal bars with roasted rice bran levels between 10 and 20% were accepted by consumers.

Zhenget *al.* (2012) developed the white rice bran, a byproduct of rice milling, was developed for L-lactic acid production. The hydrolyte of white rice bran could be used as carbon and nutrient sources for L-lactic acid fermentation with a productivity of $3.73 \text{ kg m}^{-3} \text{ h}^{-1}$ in batch fermentation, which was 1.52 times higher than that of the control medium with glucose and yeast extract. These results showed a potential for development of high value-added products from white rice bran.

Salehi and Bibalan (2012) studied on utilization of indigenous rice bran (RB) for the preparation of value-added products. The stabilized rice bran is used to determine the effect of dough rheological and muffin cake sensory properties of one type of wheat flour (with 75% extraction rate). Water absorption, dough development time (30% rice bran) and calorimetric value were increased and dough stability and dough softening were decreased by addition of rice bran in flour than the control. Sensory evaluation with Friedman test revealed that there is no significant difference between treatments at 5% level. Cakes containing 20% rice bran flour got the highest scores for sensory evaluation. It was concluded that the quantity and quality rheological and sensory properties of muffin cakes was improved with the addition of rice bran flour. Thus, rice bran could be used for wheat substituting and a good functional ingredient for value addition of food products. Moreover the present study suggests that T3 (30% RB + 70% wheat flour) can produce superior quality cakes to prove effectiveness of RB as bakery powder.

Yadav *et al.* (2012) investigated the possibility of utilizing defatted rice bran (DRB) for making chapati. DRB was ground and blended with wheat flour in the proportion of 0, 5, 10, 15, 20 and 25% blends were evaluated for dough and chapati making quality. Extensibility of dough and chapati decreased (19.9 ± 0.08 mm to 14.3 ± 0.08 mm, 11.2 ± 0.05 mm to 6.3 ± 0.04 mm, respectively) while peak load to rupture of chapatti increased (3.1 ± 0.04 to 3.6 ± 0.05 N) at 25% replacement of flour with fine DRB. Water absorption capacity of flour increased from $73 \pm 0.4\%$ to $74.6 \pm 0.4\%$ with 25% of large DRB, while the increase was up to 78.4 ± 0.3 and $80.6 \pm 0.3\%$ in case of medium and fine DRB. Overall acceptability score of chapati was also significantly decreased (8.4 ± 0.2 to 6.8 ± 0.2) with 20% of large DRB, whereas it was 7.0 ± 0.1 and 7.6 ± 0.1 for medium and fine DRB at the same proportion. The chapati prepared from the wheat flour containing 20% fine DRB was rated acceptable and also had significantly higher ash ($2.1 \pm 0.05\%$) and total dietary fiber ($4.3 \pm 0.10\%$) content than control chapati.

Premakumari *et al.* (2012) developed the breakfast / dinner recipes by substituting cereals with rice bran at different levels and determined the acceptability of food mixes. Stabilization studies revealed that microwave stabilized parboiled rice bran had low moisture and low free fatty acid content and hence considered ideal for product development. Ten standard Indian subcontinent breakfast/dinner recipes namely chapati, mixed vegetable chapati, wheat dosa, wheat rava idly, adai, ravaadai,

ragiadaai, rice vermicelli, ragi vermicelli and kolukattai were chosen for incorporating rice bran at 25, 30 and 35% replacing the cereals and pulses in the standard recipe. The acceptability trials were carried out using 20 semi trained panel members. The results revealed that, the recipes with 25% incorporation of rice bran had a good acceptability and on par with standard recipes.

Bhaduri (2013) conducted study to produce gluten free healthy cereal based muffins prepared from two gluten free flours, rice and quinoa flour. 100% wheat flour was used as control. Rice flour was replaced by 25, 50, 75 and 100% quinoa flour to prepare, muffin. Physical property measurements including percentage increase/decrease of crest height, moisture and specific gravity, color and texture analyzer was done for the final product. The sensory attributes, appearance, flavor, sweetness, texture and general acceptability, were evaluated by using a 9-point hedonic scale. The study showed that, 100% rice flour and 25 to 75% replacement with quinoa flour to rice flour formulations for muffin has the better overall consumer acceptability compared to 100% quinoa flour muffin.

Amehet *al.* (2013) studied the effect of rice bran supplementation on physico-chemical and sensory properties of wheat bread. Blends of wheat flour and rice bran (95:5, 90:10 and 85:15) were used to bake bread with 100% wheat flour as control. Thereafter, proximate, vitamin and mineral composition, as well as the physical and sensory properties of the dough and bread loaves were determined, using standard methods of analysis. The moisture content, crude protein, crude fat, crude fiber, and ash of the composite bread loaves increased significantly, while carbohydrate content decreased with increased level of supplementation. Bread loaf weight increased while loaf volume and specific loaf volume decreased. There was a significant difference in physical properties of dough and bread loaves between the composite bread and the control. Hence, it is concluded that 95:5 blend was better accepted compared to the other blends and there was a significant improvement in the nutritional composition of the wheat bread with rice bran supplementation.

Bariset *al.* (2014) evaluated the Infrared stabilized rice bran (SRB) substitution with the whole grain wheat breads at the levels of 2.5, 5.0 and 10.0% in terms of proximate composition, crumb color, dietary fiber, texture and sensory attributes. An increasing tendency was observed in crude fat and ash content of the breads. Redness (a*), yellowness

(b*) chroma and redness (a*/b*) values were increased gradually with the addition of SRB. Crumb color was found to be darker when 10% of SRB was added to the breads. SRB inclusion did not affect the content of soluble dietary fiber while it significantly increased the insoluble and total dietary fiber contents of the breads.

2.5 Shelf life study of the developed products

Delahaye *et al.* (2004) incorporated stabilized rice bran flour (SRBF) with a dietary fiber content of 26% in the production of pizza dough. The pizza dough was developed mixing wheat flour with SRBF in a proportion of 5:95 and 10:90; SRBF: wheat flour, water, salt, and yeast. All the pizzas were stored for 60 days at -18°C and their proximal composition, functional properties, and sensorial characteristics were evaluated at 0, 30 and 60 days. The results showed that the content of dietary fiber increased to 3.8% and 5.3% as the level of enrichment increased. The farinographic curves of the pizza dough showed that the development time, water absorption, and stability decreased, while mixing tolerance index and departure time were not affected by enrichment level. During storage (60 days), starch content of the three flours was decreased. The sensorial test results indicate that the pizza dough with an enrichment level of 5% with SRBF was well accepted by the panel and it was stable up to 60 days at -18°C.

Ajmalet *al.* (2006) incorporated defatted rice bran (DRB) at different levels with wheat flour. Five treatments (TO = control i.e. without DRB; T5 = 5 % DRB; T10 = 10 % DRB; T15 = 15% DRB; T20 = 20% DRB) were used for bread preparation. Bread loaves were analyzed for chemical composition and sensory evaluation at different storage intervals i.e. S0, S24, S48, S72, S96, and S120 hours. Protein, ash, fiber, and mineral contents of breads were improved and moisture decreased significantly, whereas fat content showed non-significant effect for increasing levels of defatted rice bran. Maximum protein, ash, fiber, K, Ca, and Mg contents were found in T20 while minimum values were observed in TO. Moisture and Na contents were decreased by the subsequent addition of rice bran. Treatment T5 got maximum scores for external characteristics (volume, color of crust, symmetry of form, evenness of bake, character of crust) and internal characteristics (grain, color of crumb, aroma, taste, and texture) of pan bread. From chemical assay and sensory evaluations, the authors concluded that the

quality bread can be improved by the addition of 5% DRB having high fiber and mineral content for commercialization.

Sharifet *al.*(2009) studied the effect of supplementation of microwave stabilized defatted rice bran in commercial straight grade wheat flout @ 10, 20, 30, 40 and 50% supplementation level to prepare fiber and mineral enriched cookies. Cookies were analyzed for physical analysis, dietary fiber, mineral content (Na, K, Ca and Mg) and sensory attributes to find out the most suitable compositions for commercialization. Overall, rice bran supplementation improved dietary fiber content and mineral profile of the cookies. On the basis of physical analysis and sensory attributes, it was concluded that defatted rice bran can be substituted up to 10 to 20% in wheat flour to prepare rice bran supplemented cookies without adversely affecting quality attributes and comparatively free from microbial spoilage and long shelf life of the product.

Nagiet *al.* (2012) formulated nutritive biscuits by using both full fat and defatted cereal bran with wheat flour at different levels. Product making, sensory and texture quality were assessed to find out the most appropriate level of bran incorporation. On the basis of quality 20 % level was selected best. Acceptability of enriched biscuits was affected with progressive storage. However, the product remained in high acceptability range up to 3 months. Free fatty acid content of biscuits was within permissible limits after 3 months of storage except rice bran (full fat) biscuits. Packaging material had significant impact on biscuit quality. The biscuits were stored safely in both packaging material i.e. HDPE and laminate. Microbiological study depicted that microbial count was far below the permissible limits up to 3 months of storage of biscuits in HDPE and laminate at room temperature. Economics of enriched biscuits revealed that wheat bran enriched biscuits were economically profitable.

Chapter III

MATERIAL AND METHODS

The chapter deals with the details of materials used and methods employed on the research topic entitled “Development and evaluation of stabilized rice bran incorporated baked products”. The present study was carried out on stabilization of rice bran and preparation of baked products from whole rice bran and stabilized whole rice bran to determine their acceptability of the baked products. The work was carried out at the Department of PGRC, PJTSAU, Hyderabad during the year 2016.

This chapter includes detailed description of experimental procedure of the study under the following heads.

3.1 Procurement of raw material

3.2 Production of rice bran

3.3 Stabilization of rice bran

3.4 Functional quality characteristics of whole rice bran and stabilized whole rice bran

3.5 Color quality of rice bran

3.6 Standardization and development of baked products (biscuits, cookies and cakes)

3.7 Sensory evaluation of the developed baked products

3.8 Physical quality characteristics of developed products

3.9 Assessment of chemical and nutritional quality characteristics of whole rice bran, stabilized whole rice bran and baked products

3.10 Assessment of microbial quality of the developed baked products

3.11 Statistical analysis of the data

3.1 Procurement of raw materials

Raw materials required for baked products development namely wheat flour and other ingredients such as fat, milk, salt and sugar were procured from the local market, Hyderabad. Cleaned and dried paddy (*Oryza sativa*) of the variety RNR 15048 were procured from ARI Rajendranagar, Hyderabad. Chemicals and glassware were utilized from the laboratory of PGRC, PJTSAU, Hyderabad.

3.2 Production of rice bran

A sample of 12 kg at 14% moisture content was dehused by a rubber rollhuller. The mixture comprising brown rice and paddy was separated by a paddy separator. Brown rice (approximately 9 kg) obtained from the paddy separator was milled by a horizontal abrasive whitener. The bran was produced from brown rice that was milled at 4% and 8% milling degree (MD). These brans were produced through adjustments of a steel weight-load and flow rates (Wahid *et al.*, 1997). Milling degrees at 4% and 8% were chosen for the purpose of the study as earlier study (Rosniyana *et al.*, 2005) had indicated that rice bran produced at 4% was the most nutritious compared to brans produced at other milling degrees. The flow diagram for production of rice bran is given in Fig 3.1

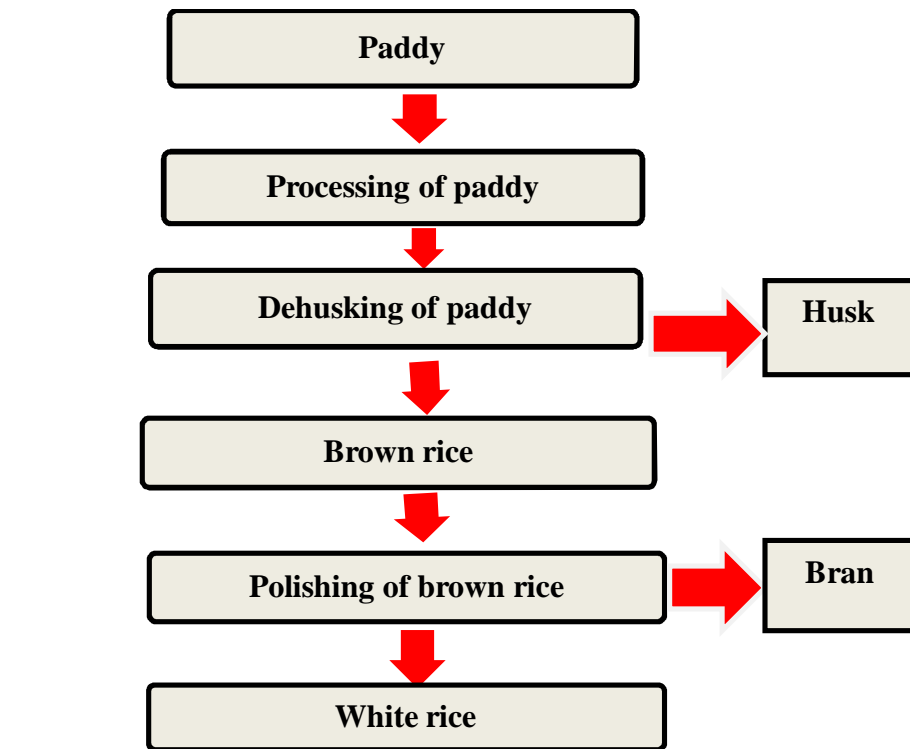


Fig. 3.1: Flow chart for production of rice bran



Plate 3.1 Production of rice bran



Plate 3.2 Whole rice bran

3.3 Stabilization of rice bran

Rice bran sample was sieved to get uniform particle size. Different substances like husk, clay and wood may be present in the bran. Hence, the full fatted raw bran was sieved through sieve number 22, which removes husk, clay, wood and saw dust. The samples thus obtained were free from impurities.

100 grams of rice bran sample was taken and microwave stabilization was done for 3 minutes to inactivate the lipase enzyme (Tao *et al.*, 1993). Flow diagram for stabilization of rice bran is given in Fig 3.2.



Plate 3.3 Stabilization of rice bran



Plate 3.4 Stabilized whole rice bran

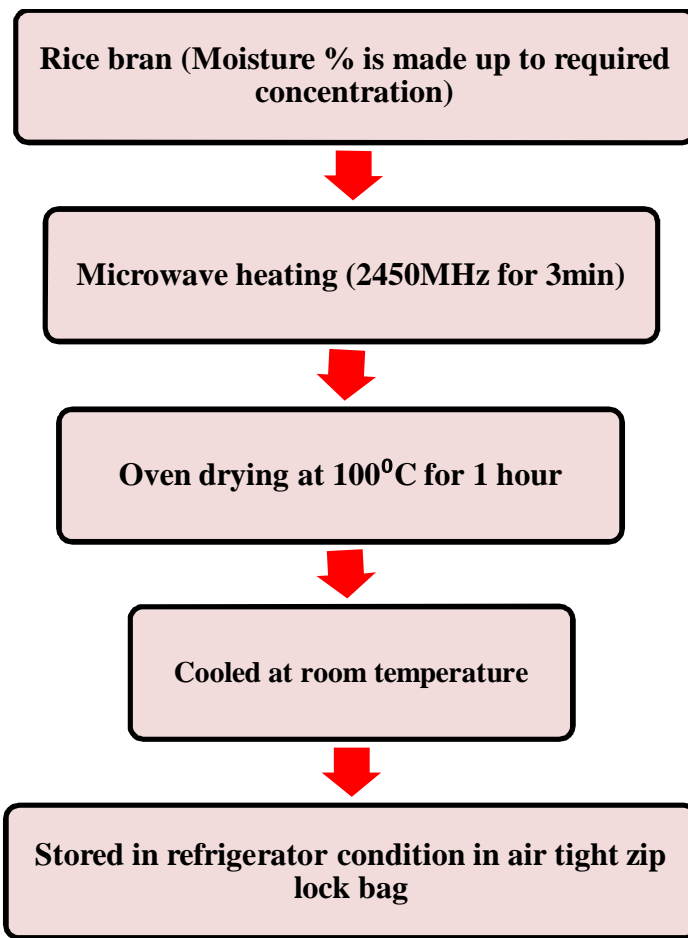


Fig. 3.2: Flow chart for stabilization of rice bran

3.4 Functional quality characteristics of WRB and SWRB

3.4.1 Estimation of bulk density (Mohsenin, 1986)

Centrifuge tube was weighed and flour sample were filled to 10 ml mark by constant tapping, until there was no further change in volume. The content was weighed and from the difference in weight, the bulk density of sample was calculated and the result was expressed as g/ml.

3.4.2 Estimation of water absorption capacity (Mohsenin, 1986)

Ground extrudate was suspended in water at room temperature for 30 min. Gently stirred during this period and centrifuge for 15 min. Supernatant was decanted into an evaporating dish of known weight.

$$\text{WAC} = \frac{\text{Weight of sediment}}{\text{Weight of dry solids}}$$

3.5 Color quality of WRB and SWRB (Hunter Lab, 2013)

Color quality of the samples was estimated by using Hunter lab calorimeter (Color Quest XE Hunter Lab, USA). Color lab scale values (CIE LAB scale) were determined by using hunter calorimeter. L* indicates lightness and extends from 0.0 (black) to 100.0 (white). The other two coordinates a* and b* represent redness (+a*value) to greenness (-a*value) and yellowness(+b*value) to blueness(-b*value).

3.6 Standardization and development of baked products

The baked products namely biscuit, cookies and cake were developed by incorporation of whole rice bran and stabilized whole rice bran at three different levels *i.e.* 10%, 20% and 30%. Products without the addition of rice bran served as a control.

Totally six variations were formulated by incorporating two types of rice bran. The preparation procedure of baked products (biscuits, cookies and cakes) in detail is given in Appendix-I, Appendix-II and Appendix-III. Baked products were prepared by using the recipe given in AACC (2000). Flow diagram for preparation of baked products such as biscuits, cookies and cakes is given in Fig 3.3, Fig 3.4 and Fig 3.5

Table 3.1 Formulations used for the development of baked products

Variation	Wheat flour (%)	WRB (%)	SWRB (%)
C	100	0	0
T1	90	10	0
T2	90	0	10
T3	80	20	0
T4	80	0	20
T5	70	30	0
T6	70	0	30

Note: WRB- whole rice bran and SWRB- stabilized whole rice bran.

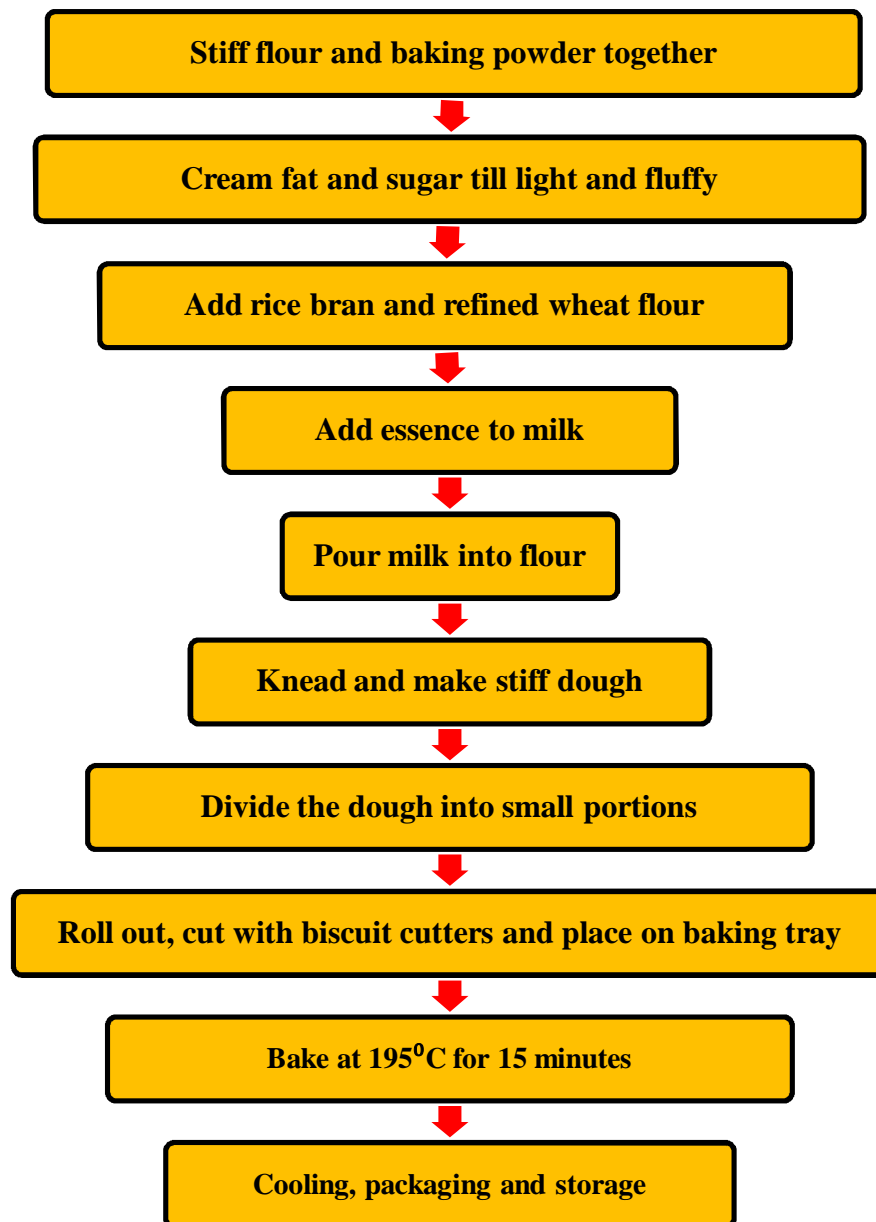


Fig. 3.3: Flow chart for preparation of biscuits



Plate 3.5.a Control

Plate 3.5.b WRB 10%

Plate 3.5.c SWRB 10%

Plate 3.5 WRB and SWRB incorporated biscuits

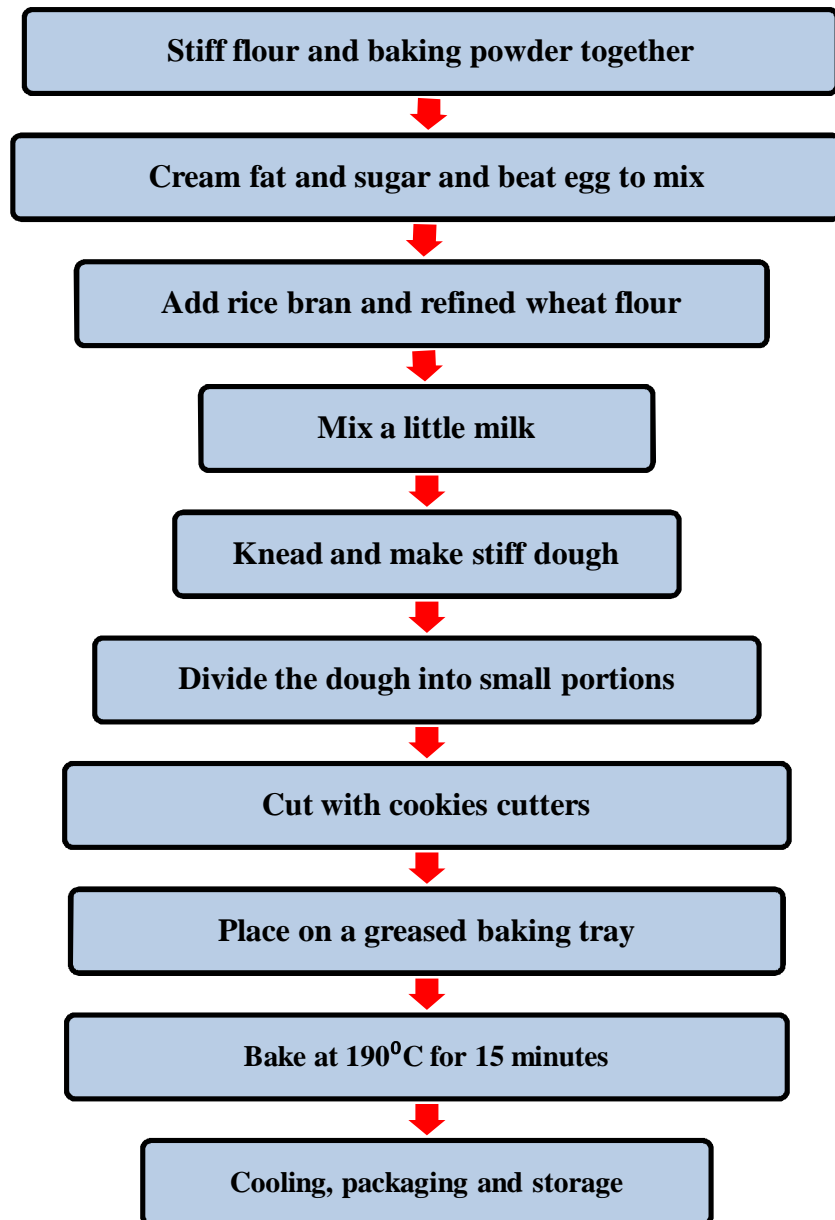


Fig. 3.4: Flow chart for preparation of cookies



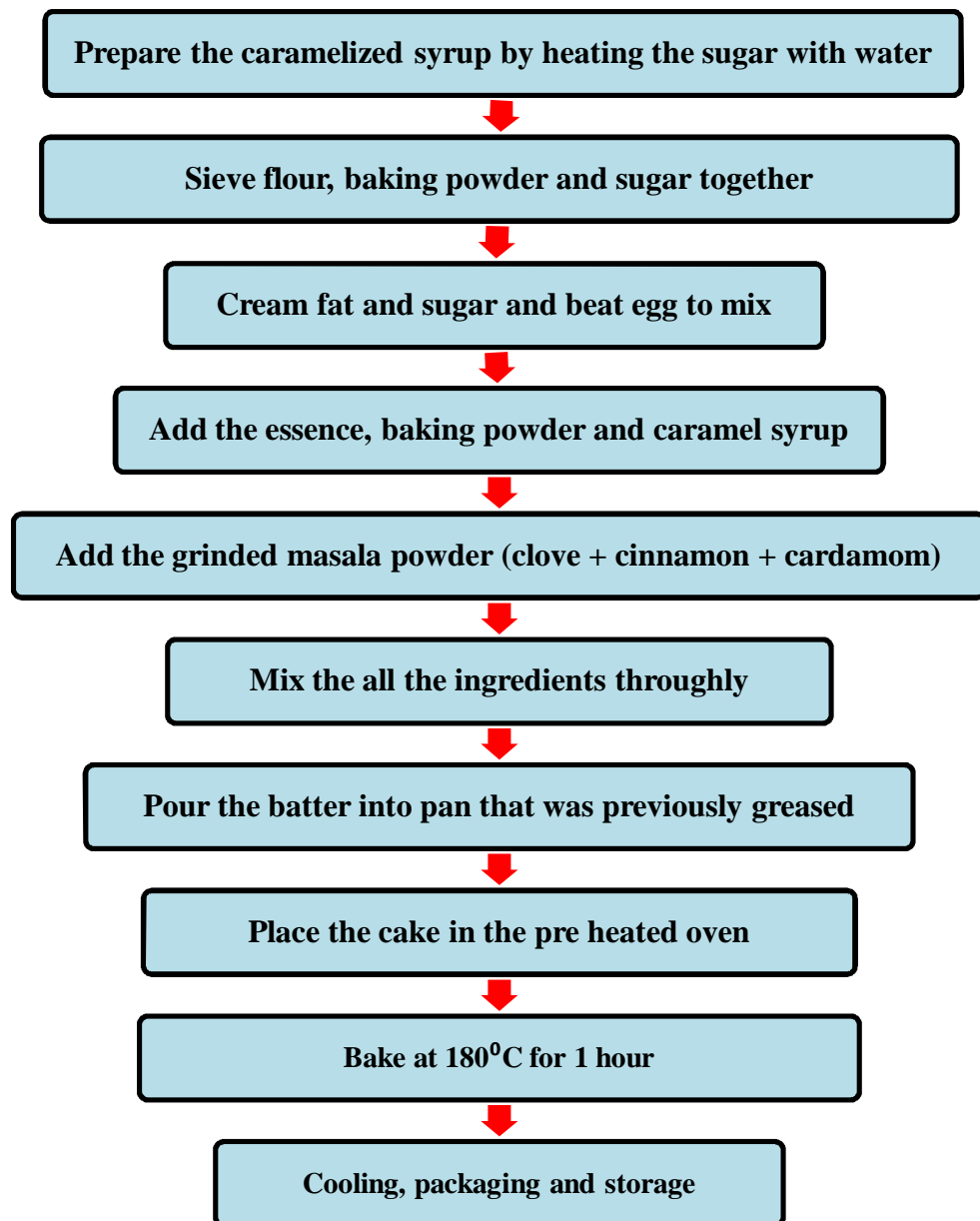


Fig. 3.5: Flow chart for preparation of cakes

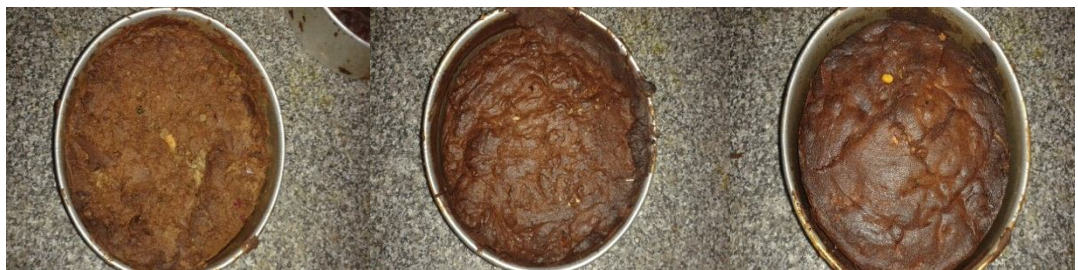


Plate 3.7.a Control

Plate 3.7.b WRB 10%

Plate 3.7.c SWRB 10%

Plate 3.7 WRB and SWRB incorporated cakes

3.7 Sensory evaluation of the developed baked products

The baked products prepared by manual method with refined wheat flour and rice bran were studied for sensory characteristics. Semi trained panel members at post graduate and research centre, Rajendranagar, Hyderabad, evaluated of baked products using 9 point hedonic scale. Samples were scored for color, texture, flavor, taste, and overall acceptability. Scores were based on a hedonic scale of 1 to 9 where: 1=I Dislike extremely (very bad) and 9 = I Like extremely (excellent), (Meilgaard *et al.*, 1999).The score card for evaluation of sensory properties of the products is given in Appendix-IV.



Plate 3.8 Sensory evaluation of baked products

3.8.Physical quality characteristics of developed products

Physical quality characteristics like diameter, thickness and spread factor were determined according to the (AACC, 2000) method.

3.8.1 Diameter of biscuits and cookies

The cookies and biscuits were selected randomly, weighed using analytical balance and the height and diameter were measured with a caliper. To measure the diameter of cookies and biscuits, four samples were placed next to one another and the total diameter was measured. All of them were then rotated at 90° and the new diameter was measured.

3.8.2 Thickness of biscuits and cookies

Thickness as measured by stacking the cookies and biscuits one above the other and restacking four times and the new thickness was measured.

3.8.3 Spread factor of biscuits and cookies

Spread factor was calculated according to the formula given below.

$$SF = (W/T \times CF) \times 10$$

Where, CF= Correlation factor (1.0 in this case)

SF = Spread factor

W = Width of the product

T = Thickness of the product

3.8.4 Physical quality characteristics of cakes

The height, length and width of the rectangular shaped cakes were measured with vernier caliper. Weight was determined with digital weighing balance. Volume of cake was calculated as length \times width \times height. Density was calculated as weight / volume.

3.9 Assessment of chemical and nutritional quality characteristics of whole rice bran, stabilized whole rice bran and baked products

The whole rice bran, stabilized whole rice bran and baked products were subjected to nutrient analysis, where the rice bran was taken for macro and micro nutrient analysis. Macronutrients such as moisture, protein, fat, ash, carbohydrate and crude fiber were analyzed using the standard procedure. Micronutrients such as calcium, and iron content of rice bran were analyzed by standard procedure of Association of Official Analytical Chemists (AOAC, 2000).

3.9.1 Estimation of moisture (AOAC, 1990)

Moisture was determined by taking 10 g of sample in petridis and dried in an oven at 1050C till the weight of the petridish with its content was constant. Each time before weighing, the petridish was cooled in desiccators. Moisture content was calculated according to the formula given below. The procedure in detail is given in Appendix-V.

$$\text{Moisture (\%)} = \frac{(W_2 - W_1) - (W_2 - W_3)}{(W_2 - W_1)} \times 100$$

Where,

W_1 = Initial weight of petridish (g)

W_2 = Weight of the petridish with sample before drying (g)

W_3 = Weight of the petridish with sample after drying (g)

3.9.2 Estimation of protein (AOAC, 2005)

The protein content of the dried samples was estimated as per cent total nitrogen by the Micro-kjeldahl procedure. Protein per cent was calculated by multiplying the per cent nitrogen by the factor 6.25. Protein content was calculated according to the formula given below. The procedure in detail is given in Appendix-VI.

Titre value \times Normality of HCL \times 14.001 \times 6.25

$$\text{Percent of protein} = \frac{\text{Titre value} \times \text{Normality of HCL} \times 14.001 \times 6.25}{\text{Weight of the sample}} \times 100$$

3.9.3 Estimation of fat (AOAC, 1981)

Fat was estimated as crude ether extract using moisture free sample. The solvent was removed by evaporation and the residue of fat was weighed. Fat content was calculated according to the formula given below. The procedure in detail is given in Appendix-VII.

$$\text{Fat content of the sample (g \%)} = \frac{W_2 - W_3}{W_1} \times 100$$

Where,

W_1 = Weight of sample

W_2 = Weight of dried extraction cup before fat extraction

W_3 = Weight of dried extraction cup after fat extraction

3.9.4 Estimation of crude fibre (AOAC, 1990)

Crude fibre of the sample was estimated by using moisture and fat free samples and expressed as g/100g of the sample. The procedure in detail is given in Appendix-VIII.

$$\text{Crude fibre (\%)} = \frac{100 - (\text{moisture} + \text{fat}) \times \text{Weight of the crude fibre}}{\text{Weight of sample taken (Moisture and fat free) (W}_1\text{)}}$$

Where,

Weight of the sample = (W_1) g

Weight of the crucible + sample before heating at 600°C = (W₂) g

Weight of the crucible + sample after heating at 600°C = (W₃) g

Weight of crude fibre = (W₂-W₃) g

3.9.5 Estimation of total ash (AOAC, 1984)

The ash content of sample was obtained by dry ashing the samples completely by heating it over a flame. This was expressed as g/100g of the sample. Ash content was calculated according to the formula given below. The procedure in detail is given in Appendix-IX.

$$\text{Ash \%} = \frac{\text{weight of the ash (w}_3\text{-w}_1\text{)}}{\text{Weight of the sample taken (w}_2\text{-w}_1\text{)}} \times 100$$

Where,

Weight of the ash= w₃-w₁

Weight of the sample taken= w₂-w₁

3.9.6 Estimation of carbohydrate (AOAC, 1981)

The carbohydrate content of samples was analysed by standard procedure of AOAC, (1981) using Anthrone method. The procedure in detail is given in Appendix-X

3.9.7 Estimation of calcium in WRB and SWRB (AOAC, 2000)

The calcium content of the samples was analysed by standard procedure of AOAC, (2000). The procedure in detail is given in Appendix-XI

3.9.8 Estimation of iron in WRB and SWRB (AOAC, 2000)

The iron content of the samples was analysed by standard procedure of AOAC, (2000). The procedure in detail is given in Appendix-XII.



Plate 3.9 Estimation of moisture content



Plate 3.10 Estimation of ash content



Plate 3.11 Analysis of protein content

3.10 Assessment of microbial quality of the developed baked products

The microbiological analysis of the developed baked products were carried out as per the standard method, for bacteria Nutrient Agar was used and for Fungi using Rose Bengal Agar (RBA). Ten gram of sample was diluted in 90 ml of buffer blanks and subsequent dilutions were prepared up to 10^{-6} dilution. Two dilution factors were used for plating of bacteria and fungi viz. 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} . The total number of microbial count was calculated using the formula given below (Thambekaret al., 2009).

$$\text{No. of colonies} \times \text{dilution factor} \\ \text{No. of microorganisms (per g/ml)} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Weight / volume of aliquots taken (g/ml)}}$$



Plate 3.12 Sterilization of the media



Plate 3.13 Incubation of the sample



Plate 3.14 Microbial analysis under laminar air flow chamber

3.11 Statistical analysis

All the result was statistically analyzed to test the significance of the results using percentages, means, standard deviations and analysis of variance (ANOVA) technique. (Snedecor and Cochran, 1980).

Chapter IV

RESULTS AND DISCUSSION

The present study was planned to study the development and evaluation of stabilized rice bran incorporated baked products. The data was subjected to statistical analysis and the results obtained are presented in this chapter. The results obtained have been presented under following sections:

4.1 Nutritional quality characteristics of rice bran

4.2 Functional quality characteristics of rice bran

4.3 Color quality of rice bran

4.4 Sensory quality characteristics of baked products

4.5 Physico-chemical and nutritional quality characteristics of baked products

4.5.1 Physical quality characteristics of baked products

4.5.1.1 Physical quality characteristics of biscuits

4.5.1.2 Physical quality characteristics of cookies

4.5.1.3 Physical quality characteristics of cakes

4.5.2 Chemical and nutritional quality characteristics of baked products

4.5.2.1 Chemical and nutritional quality characteristics of biscuits

4.5.2.2 Chemical and nutritional quality characteristics of cookies

4.5.2.3 Chemical and nutritional quality characteristics of cakes

4.6 Shelf life studies of baked products

4.7 Microbial quality of baked products

4.1. Nutritional quality characteristics of rice bran

Nutrient composition of whole rice bran (WRB) and stabilized whole rice bran (SWRB) samples are shown in Table 4.1 and graphically as shown in Fig 4.1. The moisture content was 10.77% and 7.11%, protein content was 15.66% and 16.28%, ash content was 9.63% and 8.29%, crude fibre content was 9.03% and 8.07%, fat content was 20.47% and 19.11% and carbohydrate content was 38.62% and 34.41%, calcium content was 5.05% and 5.415% and iron content was 0.546% and 0.705% in whole rice bran and stabilized whole rice bran respectively.

The moisture value was significantly higher ($P < 0.05$) for the whole rice bran (10.77%) compared to the stabilized whole rice bran (7.11%). Significant difference was not found in the protein and calcium content between the whole rice bran and stabilized whole rice bran. Statistically significant difference was observed in moisture, ash, fiber, fat, carbohydrate and iron content of WRB and SWRB.

The micronutrient composition of stabilized rice bran was similar to the result reported by Rabbani and Ali (2009) where in 4.82% of calcium and 0.52% of iron was found in rice bran samples.

Table 4.1: Nutritional quality characteristics of rice bran

Nutrients	WRB	SWRB	CD value
Moisture (%)	10.77 ^a ±0.30	7.11 ^b ±0.17	0.564
Protein (%)	15.66 ^a ±0.23	16.28 ^a ±0.29	0.597
Ash (%)	9.63 ^a ±0.18	8.29 ^b ±0.37	0.661
Crude fiber (%)	9.03 ^a ±0.09	8.07 ^b ±0.20	0.363
Fat (%)	20.47 ^a ±0.54	19.11 ^b ±0.40	1.093
Carbohydrate (%)	38.62 ^a ±2.16	34.41 ^b ±0.94	3.782
Calcium (mg/100g)	50.57 ^a ±1.15	54.15 ^a ±1.32	2.816
Iron (mg/100g)	5.46 ^a ±0.79	7.05 ^b ±0.19	1.308

Note:

- WRB- Whole rice bran.
- SWRB- Stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

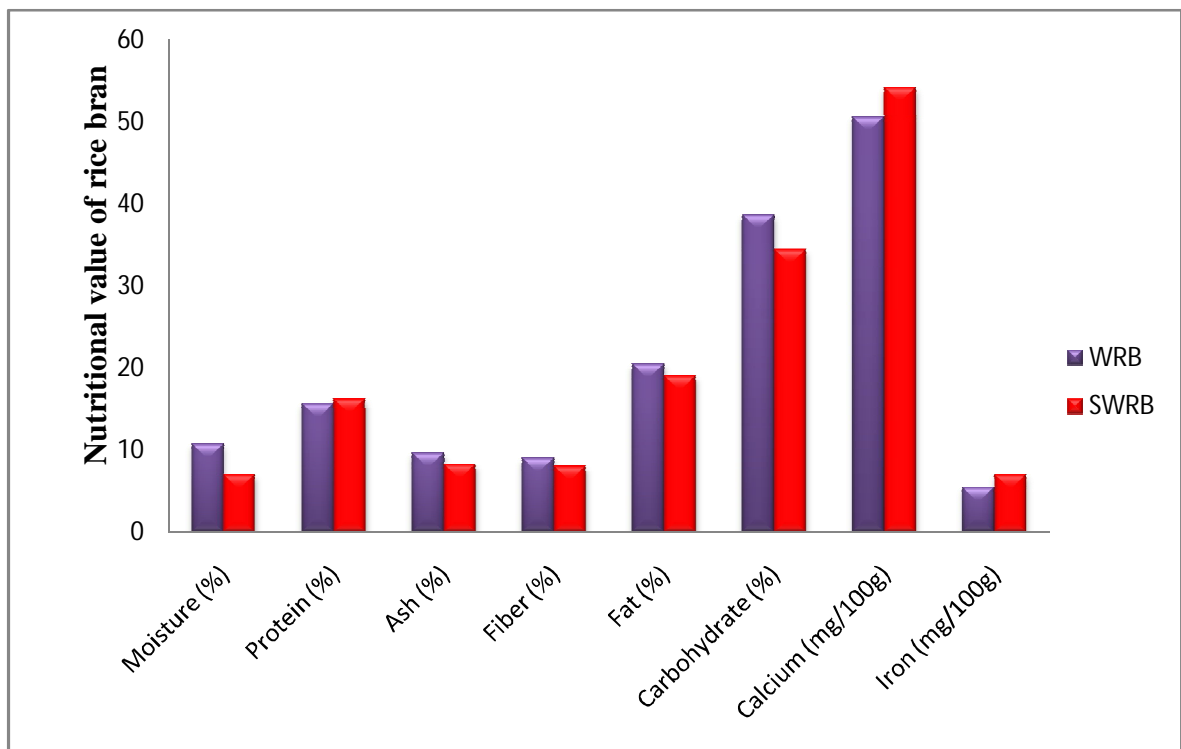


Fig 4.1 Nutrient content of whole rice bran in comparison with stabilized whole rice bran

4.2. Functional quality characteristics of rice bran

The functional properties analyzed for the whole rice bran and stabilized whole rice bran were bulk density and water absorption as shown in Table 4.2 and graphically as shown in Fig 4.2.

4.2.1 Bulk density

The bulk density was 0.31g/ml and 0.24g/ml for the whole rice bran and stabilized whole rice bran respectively.

Statistically significant difference was observed in bulk density between the whole rice bran and stabilized whole rice bran.

Each type or variety of grain has a characteristic bulk density. This is defined as the weight per standard volume, measured in a standard manner. In the present study the bulk density of whole rice bran was higher when compared to stabilized whole rice bran *i.e.* 0.31g/ml and for stabilized whole rice bran was 0.24g/ml. The results are on par with the reported values by Chandi and Sogi (2006) study in which it was reported that the bulk density of basmati rice bran samples was 0.21g/ml.

4.2.2 Water absorption capacity

The water absorption was 2.17ml/g and 2.33ml/g for whole rice bran and stabilized whole rice bran respectively.

Statistically no significant difference was observed in the water absorption value between the whole rice bran and stabilized whole rice bran.

Water absorption capacity is kinetics of water movement under controlled condition. The amount of water retained by the solids was measured. Dietary fibre present in the bran is known to bind water. The results are in harmony with the results obtained by Sairamet *al.*, (2011) who reported that the water absorption values found between 2.0 to 2.60ml/g in the defatted rice bran samples.

Table 4.2: Functional quality characteristics of rice bran

Samples	Bulk density (g/ml)	Water absorption (ml/g)
WRB	0.31 ^a ±0.03	2.17 ^a ±0.28
SWRB	0.24 ^b ±0.02	2.33 ^a ±0.29
CD values	1.742	0.654

Note:

- WRB- Whole rice bran.
- SWRB- Stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

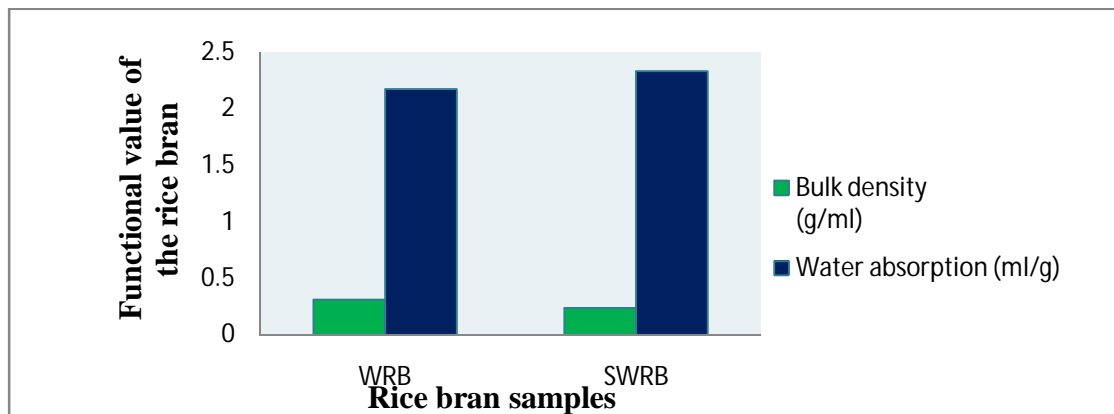


Fig 4.2 Functional quality characteristics of whole rice bran in comparison with stabilized whole rice bran

4.3 Color values of rice bran

The results of color scores of the rice bran is presented as L*, a* and b* values and given in Table 4.3 and graphically as shown in Fig 4.3. The L* value ranged from 0 to 100 indicating luminance or lightness component along with two chromatic components a* component (from green to red) and the b* component (from blue to yellow). The L*a*b* units are often used in food research studies because of uniform distribution of colors and as L*a*b* units are very close to human perception of color.

The L* value was 62.77 ± 0.44 and 58.73 ± 0.78 for the whole rice bran and stabilized whole rice bran respectively.

The a* value was 1.81 ± 0.24 and 2.48 ± 0.15 for the whole rice bran and stabilized whole rice bran respectively.

The b* value was 18.22 ± 0.65 and 20.33 ± 0.87 for the whole rice bran and stabilized whole rice bran respectively.

The L* value was significantly higher ($P < 0.05$) for the whole rice bran compared to the stabilized whole rice bran. The a* and b* values were significantly lower ($P < 0.05$) for the whole rice bran compared to the stabilized whole rice bran.

However, increase in a* and b* values indicated that increase in redness and yellowness of bran particles, respectively. These values indicated that the stabilized whole rice bran had changed colors slightly to yellowish and reddish compared with whole rice bran sample.

Table 4.3: Color values of rice bran

Samples	Color values		
	L*	a*	b*
WRB	62.77 ^a ±0.44	1.81 ^a ±0.24	18.22 ^a ±0.65
SWRB	58.73 ^b ±0.78	2.48 ^b ±0.15	20.33 ^b ±0.87
CD values	1.444	0.467	1.742

Note:

- WRB- Whole rice bran.
- SWRB- Stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

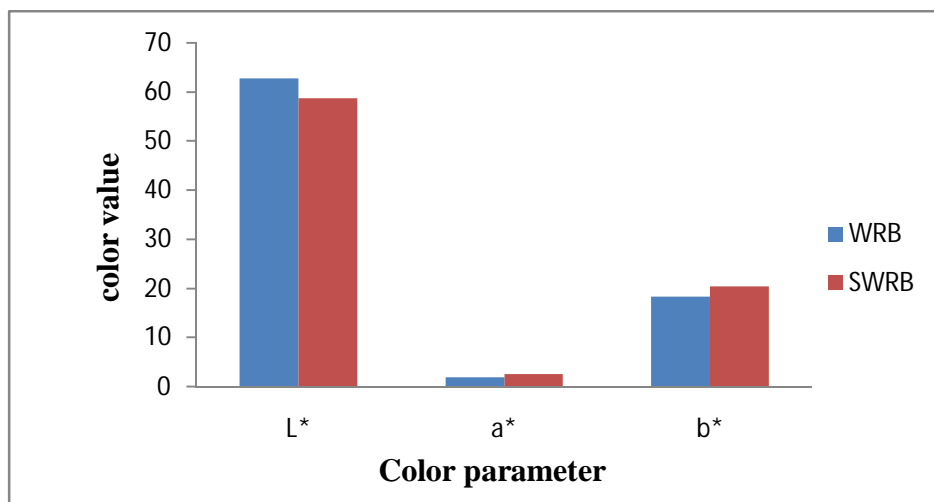


Fig 4.3 Color values of rice bran

4.4 Sensory quality characteristics of baked products

Sensory quality characteristics was carried out by using semi trained panelists to measure sensory characteristics like appearance, smell, taste, touch and acceptability of baked products (biscuit, cookies and cakes) by using 9 point hedonic scale.

4.4.1 Mean sensory scores of biscuits

Sensory evaluation of biscuits prepared with wheat flour with and without the addition of rice bran was done by semi trained panel of judges. The mean scores of sensory evaluation for the biscuits are given in Table 4.4 and graphically as shown in Fig 4.4.

Sensory rating of biscuits for color showed that control sample (7.96) ranked at top due to excellent appearance, followed by T2 (7.07), T1 (6.86), T4 (6.57) & T3 (6.36) while minimum mean sensory score of color was observed for T6 (6.29) & T5 (6.00). The mean sensory score of color declined from 7.96 to 6.00 with increasing level of substitution of rice bran. The color of biscuits turned from light brown to dark brown, leading to lower acceptance.

Mean sensory score for texture of biscuits decreased from 7.50 to 5.57 with increasing level of substitution of rice bran. Mean sensory score for texture showed in Table 4.4 revealed that the control sample had highest mean score for texture (7.50) followed by T2 (7.21), T1 (6.86), T4 (6.79) & T3 (6.43) while T6 (5.86) and T5 (5.57) had least mean score for texture.

Mean sensory score for taste of biscuits decreased from 7.50 to 5.57 with increasing level of substitution of whole rice bran and stabilized rice bran. Mean sensory score for taste as showed in Table 4.4 revealed that the control sample had highest mean score for taste (7.50) followed by T2 (6.71), T1 (6.29), T4 (6.21) and T3 (5.95) while T6 (5.93) and T5 (5.57) had least mean score for taste.

Mean sensory score for flavor of biscuits decreased from 7.50 to 5.71 with increasing level of substitution of rice bran as showed in the Table 4.4. Mean sensory score for flavor showed in Table 4.4 revealed that the mean sensory score for flavor in control (7.50) at top position followed by T2 (6.70), T1 (6.43), T4 (6.43) and T3 (6.14) while T6 (6.07) and T5 (5.71) had least mean score for flavor.

Overall acceptability was determined on the basis of sensory quality scores obtained by the evaluation of color, flavor, taste and texture of the biscuits. The mean sensory score regarding overall acceptability of biscuits showed in Table 4.4 revealed that the mean overall acceptability in control was maximum, while 30% of WRB and SWRB incorporating biscuits had lowest mean acceptability. The decrease in mean overall acceptability was due to decrease in mean sensory score of color, flavor, texture and taste scores.

Statistically significant difference ($P < 0.05$) was observed in mean color, texture, taste, flavor and overall acceptability scores of biscuits prepared from wheat flour with and without the addition of rice bran.

The T1 and T2 had highest scores for all the sensory attributes compare to other treatments. These values are similar to the value reported by Sharif *et al.*, (2009) study, in which it was reported that 10% of defatted rice bran incorporated biscuits were the best accepted products.

The darker color of the biscuits may be due to the non-enzymatic reaction (Maillard reaction) between reducing sugar molecules and lysine protein (Decker *et al.*, 2002). Rice bran is reported to be rich in lysine which produces darker shades of brown color. Browning color of bakery products like bread and biscuit might be due to caramelization, dextrinisation of starch or maillard reaction.

Table 4.4: Mean sensory scores of biscuits prepared by incorporating of rice bran

Treatments	Color	Texture	Taste	Flavor	Overall acceptability
C	7.93 ^a ±0.83	7.50 ^a ±0.85	7.50 ^a ±0.94	7.50 ^a ±0.85	7.93 ^a ±0.73
T1	6.86 ^{bd} ±0.77	6.86 ^{ab} ±0.86	6.29 ^b ±0.91	6.43 ^b ±0.85	6.36 ^b ±0.63
T2	7.07 ^b ±0.83	7.21 ^{ac} ±0.58	6.71 ^b ±0.91	6.7 ^b ±1.07	6.79 ^{bd} ±0.80
T3	6.36 ^{bc} ±0.74	6.43 ^{bd} ±1.09	5.95 ^{bc} ±0.73	6.14 ^{bc} ±0.66	6.21 ^b ±1.05
T4	6.57 ^{bc} ±0.85	6.79 ^{bc} ±1.05	6.21 ^b ±0.70	6.43 ^b ±0.76	6.57 ^b ±0.65
T5	6.00 ^c ±0.68	5.57 ^d ±0.85	5.57 ^c ±0.85	5.71 ^c ±0.61	5.64 ^c ±0.84
T6	6.29 ^{cd} ±0.61	5.86 ^d ±0.95	5.93 ^{bc} ±0.73	6.07 ^{bc} ±0.62	6.00 ^{bc} ±0.68
CD value	0.573	0.679	0.623	0.592	0.586

Note:

- C- Control sample.
- T1- Biscuit prepared by incorporated 10% of whole rice bran.
- T2- Biscuit prepared by incorporated 10% of stabilized whole rice bran.
- T3- Biscuit prepared by incorporated 20% of whole rice bran.
- T4- Biscuit prepared by incorporated 20% of stabilized whole rice bran.
- T5- Biscuit prepared by incorporated 30% of whole rice bran.
- T6- Biscuit prepared by incorporated 30% of stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

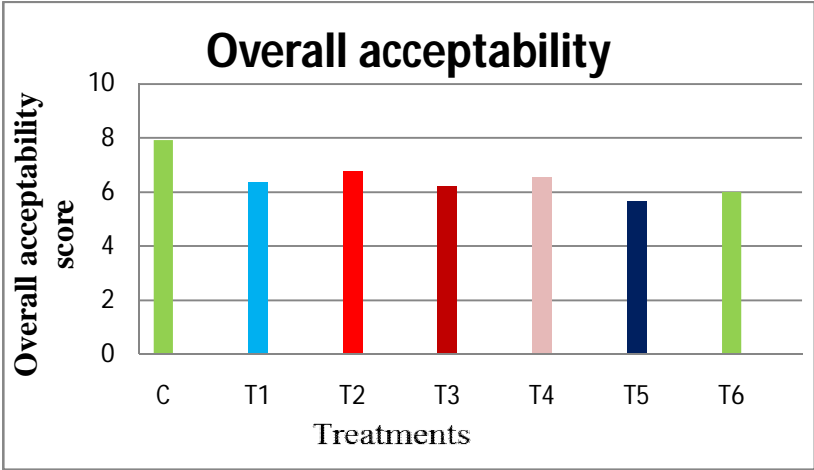
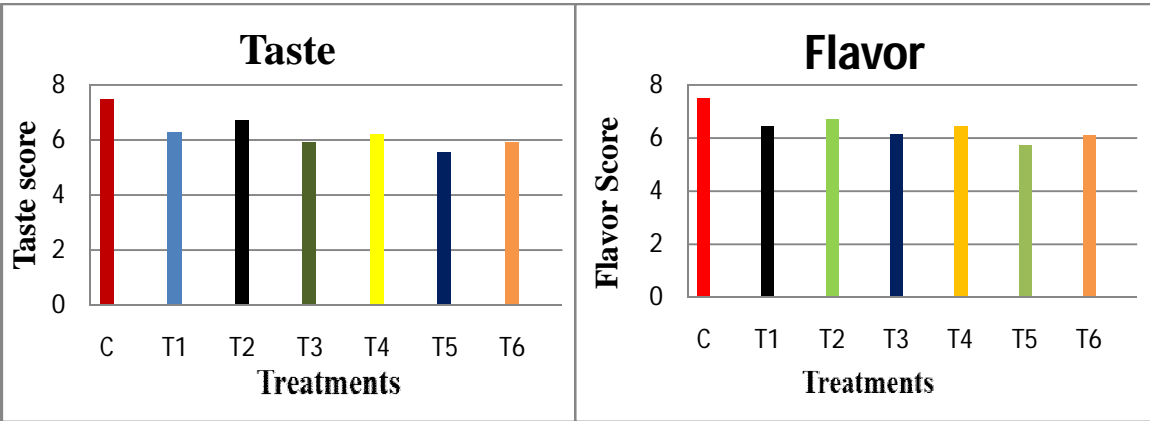
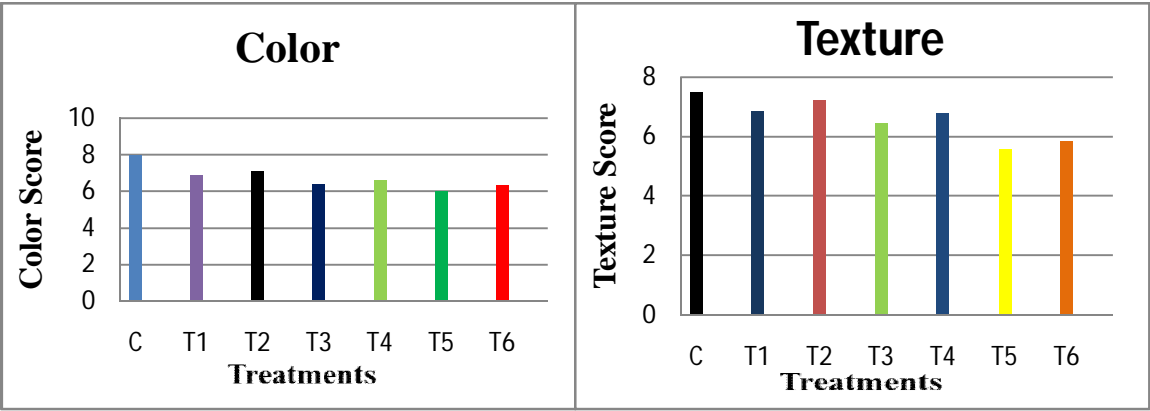


Fig 4.4 Mean sensory score of biscuits

4.4.2 Mean sensory scores of cookies

Sensory evaluation of cookies prepared with wheat flour with and without the addition of rice bran was done by semi trained panel of judges. The mean scores of sensory evaluation for the products are given in Table 4.5 and graphically as shown in Fig 4.5.

Sensory rating of cookies for color showed that control (C) got maximum score (8.00) while T6 (6.29) and T5 (5.94) obtained the lowest score. T1 (6.35), T2 (6.59), T3 (6.24) & T4 (7.06) got fairly high score which showed that color was decline from 8.00 to 6.29 with increasing level of incorporation of whole rice bran and stabilized whole rice bran. The color of cookies turned from light brown to dark brown, leading to lower acceptance.

Mean sensory score for texture of cookies decreased from 7.35 to 5.71 with increasing level of substitution of whole rice bran and stabilized whole rice bran. Mean sensory score for texture showed in Table 4.5 revealed that the control had highest mean sensory score for texture (7.35) followed by T4 (7.18), T1 (6.82), T3 (6.65) and T2 (6.59) while T6 (5.71) and T5 (5.35) had least sensory score for texture.

Mean sensory score for taste decreased from 7.59 to 5.35 with increasing level of substitution of whole rice bran and stabilized whole rice bran in cookies. Mean sensory score for taste shown in Table 4.5 revealed that the control had highest mean sensory score for taste (7.59) followed by T4 (6.94), T1 (6.12), T2 (6.12), & T3 (5.92) while T6 (5.88) and T5 (5.35) had least score for taste.

Mean sensory score for flavor of cookies decreased from 7.53 to 5.41 with increasing level of substitution of rice bran as showed in the Table 4.5. Mean sensory score for flavor showed in Table 4.5 revealed that the mean sensory score for flavor in control (7.53) at top position followed by T4 (6.71), T1 (6.42), T2 (6.35) and T6 (6.06) T3 (5.71) and T5 (5.41) had least score for flavor.

Overall acceptability was determined on the basis of sensory quality scores obtained by the evaluation of color, flavor, taste and texture of the cookies.

The mean sensory score regarding overall acceptability of cookies are showed in Table 4.5 revealed that the mean sensory score for overall acceptability in control was maximum while 30% of WRB and SWRB incorporated cookies had lowest acceptability.

The decrease in overall acceptability was due to decrease in sensory quality characteristics such as color, flavor, taste and texture scores of cookies.

Statistically significant difference ($P > 0.05$) was observed in mean color, texture, taste, flavor and overall acceptability scores of cookies prepared from wheat flour with and without the addition of rice bran.

T1 and T4 had maximum mean sensory scores for all the sensory attributes compare to other variations studied. These values are similar to the value reported by Haque *et al.*, (2002) study in which it was reported that the 10% of aqueous extracted bran has the maximum sensory scores in cookies compare to other treatments.

Table 4.5: Mean sensory scores of cookies prepared by incorporating rice bran

Treatments	Color	Texture	Taste	Flavor	Overall acceptability
C	8.00 ^a ±0.87	7.35 ^a ±0.79	7.59 ^a ±0.94	7.53 ^a ±0.87	8.00 ^a ±0.79
T1	6.35 ^b ±0.79	6.82 ^{ab} ±1.07	6.12 ^b ±1.11	6.42 ^b ±0.87	6.41 ^b ±0.69
T2	6.59 ^{bc} ±0.87	6.59 ^b ±1.18	6.12 ^b ±0.70	6.35 ^{bc} ±0.79	6.71 ^b ±0.69
T3	6.24 ^b ±0.90	6.65 ^b ±0.86	5.92 ^{bd} ±1.13	5.71 ^b ±0.92	5.65 ^c ±0.93
T4	7.06 ^c ±0.75	7.18 ^b ±0.53	6.94 ^c ±0.90	6.71 ^c ±1.10	6.71 ^b ±0.77
T5	5.94 ^b ±0.90	5.35 ^c ±1.06	5.35 ^d ±0.93	5.41 ^b ±0.80	5.24 ^c ±1.15
T6	6.29 ^b ±0.59	5.71 ^{bc} ±0.99	5.88 ^{bd} ±0.70	6.06 ^b ±0.66	5.94 ^b ±0.66
CD value	0.554	0.642	0.631	0.589	0.578

Note:

- C- Control sample.
- T1- Cookies prepared by incorporated 10% of whole rice bran.
- T2- Cookies prepared by incorporated 10% of stabilized whole rice bran.
- T3- Cookies prepared by incorporated 20% of whole rice bran.
- T4- Cookies prepared by incorporated 20% of stabilized whole rice bran.
- T5- Cookies prepared by incorporated 30% of whole rice bran.
- T6- Cookies prepared by incorporated 30% of stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

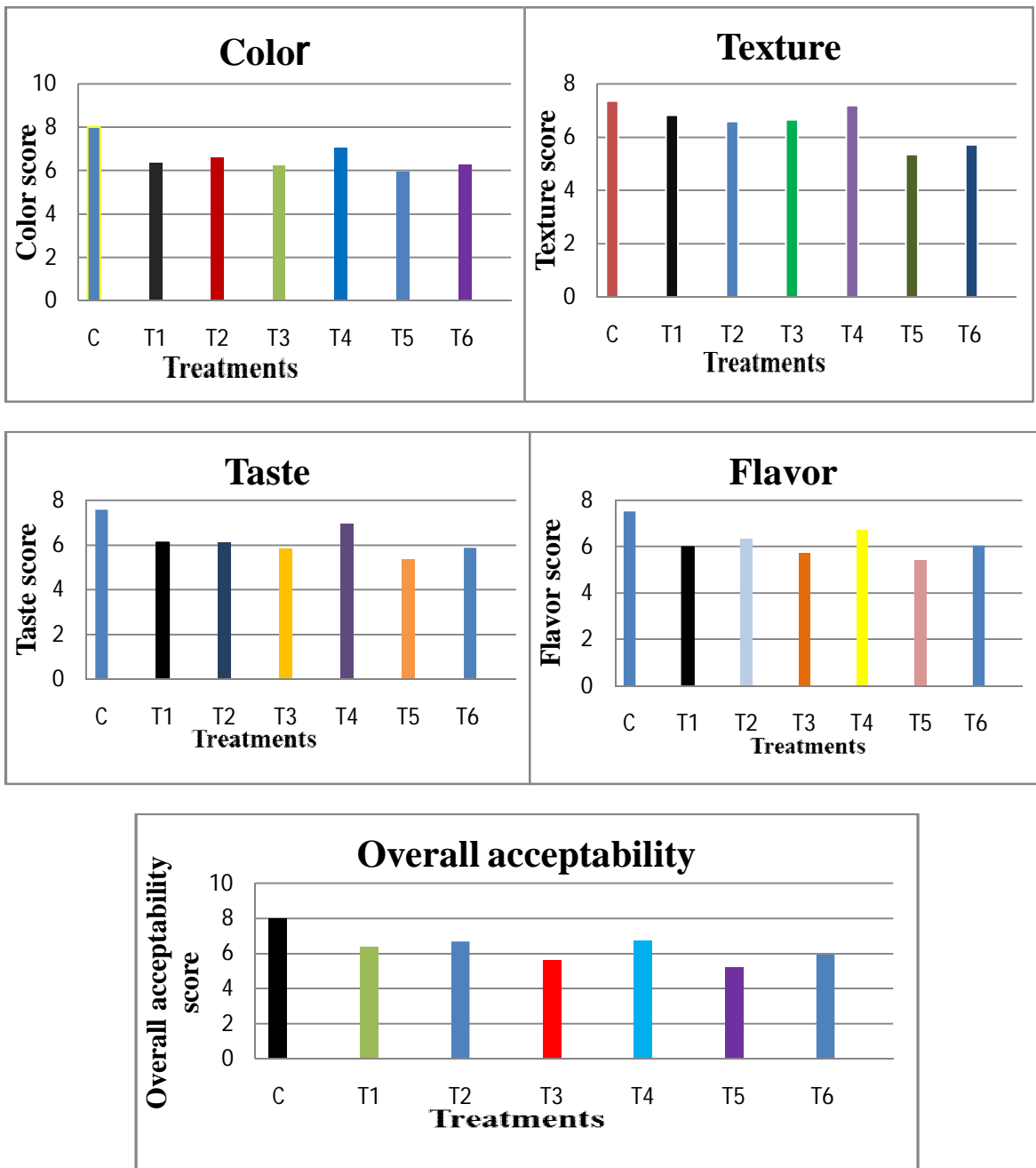


Fig 4.5 Mean sensory score of cookies

4.4.3 Mean sensory scores of cakes

Sensory quality evaluation of cakes prepared from wheat flour with and without the addition of rice bran was done by semi trained panel of judges. The mean scores of sensory evaluation for the cakes are given in Table 4.6 and graphically as shown in Fig 4.6.

The mean sensory scores for color of cake was 7.36 ± 1.22 , 6.29 ± 0.99 , 6.93 ± 0.92 , 5.43 ± 0.76 , 6.21 ± 0.89 , 4.71 ± 0.73 and 5.14 ± 0.66 for the control and T1, T2, T3, T4, T5 and T6 respectively.

The mean sensory scores for texture of cake was 7.21 ± 0.89 , 6.79 ± 0.70 , 7.21 ± 0.80 , 5.07 ± 0.73 , 5.35 ± 0.93 , 4.43 ± 0.76 and 4.92 ± 0.92 for the control and T1, T2, T3, T4, T5 and T6 respectively.

The mean sensory scores for taste of cake was 7.07 ± 1.00 , 6.00 ± 0.88 , 6.5 ± 0.76 , 5.00 ± 0.88 , 5.57 ± 0.85 , 4.86 ± 0.77 and 5.21 ± 0.89 for the control and T1, T2, T3, T4, T5 and T6 respectively.

The mean sensory scores for flavor of cake was 7.14 ± 0.86 , 5.79 ± 0.70 , 6.35 ± 1.08 , 5.36 ± 0.74 , 6.00 ± 0.78 , 4.86 ± 0.77 and 5.35 ± 0.84 for the control and T1, T2, T3, T4, T5 and T6 respectively.

The mean sensory scores for overall acceptability of cake was 7.35 ± 0.63 , 5.79 ± 0.70 , 6.42 ± 0.76 , 5.36 ± 0.93 , 6.14 ± 0.66 , 4.71 ± 0.83 and 5.35 ± 0.74 for the control and T1, T2, T3, T4, T5 and T6 respectively.

The mean sensory scores for color of the cake was maximum in control (7.36 ± 1.22) compared to all other variations studied. In whole rice bran incorporated cake the color was maximum in T1 (6.29 ± 0.92) compared to other treatments of the cake samples. In stabilized whole rice bran incorporated cake the color was maximum in T2 (6.93 ± 0.92) compared to other variations studied. T6 (5.14 ± 0.66) and T5 (4.71 ± 0.73) had least mean sensory scores for color.

The mean sensory scores for texture of the cake was maximum in control (7.21 ± 1.22) compared to other variations studied. In whole rice bran incorporated cake the texture was maximum in T1 (6.79 ± 0.70) compared to other variations studied. In stabilized whole rice bran incorporated cake the texture was maximum in T2 (7.21 ± 0.80) compared to

other variations studied. T6 (4.92 ± 0.66) and T5 (4.43 ± 0.73) had least mean scores for texture.

The mean sensory scores for taste of the cake was maximum in control (7.07 ± 1.00) compared to all other treatment of the samples. In whole rice bran incorporated cake the taste was maximum in T1 (6.00 ± 0.88) compared to other variations studied. In stabilized whole rice bran incorporated cake the taste was maximum in T2 (6.50 ± 0.76) compared to other variations studied. T6 (5.21 ± 0.66) and T5 (4.86 ± 0.77) has least mean scores for taste.

The mean sensory scores for flavor of the cake was maximum in control (7.14 ± 0.86) compared to all other variations studied. In whole rice bran incorporated cake the flavor was maximum in T1 (5.79 ± 0.70) compared to other variations studied. In stabilized whole rice bran incorporated cake the flavor was maximum in T2 (6.35 ± 1.08) compared to all other variations studied. T6 (5.36 ± 0.66) and T5 (4.86 ± 0.77) had least mean scores for flavor.

The mean sensory scores for overall acceptability of the cake was maximum in control (7.35 ± 0.63) compared to all other variations studied. In whole rice bran incorporated cake the overall acceptability was maximum in T1 (5.79 ± 0.70) compared to other variations studied. In stabilized whole rice bran incorporated cake the overall acceptability was maximum in T2 (6.42 ± 0.76) compared to other variations studied. T6 (5.35 ± 0.74) and T5 (4.71 ± 0.83) had least mean scores for overall acceptability.

Statistically significant difference ($P>0.05$) was observed in the mean scores for color, texture, taste, flavor and overall acceptability of cakes prepared from wheat flour with and without the addition of rice bran.

T1 and T2 had highest mean scores for all the sensory attributes studied compared to other variations. These values are similar to the value reported by Lebesian and Tzia (2011) study, in which it was reported that the 5-10% of edible cereal bran incorporated cupcakes had the maximum sensory scores compared to other treatments.

Table 4.6: Mean sensory scores of cakes prepared by incorporating rice bran

Treatments	Color	Texture	Taste	Flavor	Overall acceptability
C	7.36 ^a ±1.22	7.21 ^a ±0.89	7.07 ^a ±1.00	7.14 ^a ±0.86	7.35 ^a ±0.63
T1	6.29 ^b ±0.99	6.79 ^{ab} ±0.70	6.00 ^b ±0.88	5.79 ^b ±0.70	5.79 ^b ±0.70
T2	6.93 ^{ab} ±0.92	7.21 ^{ab} ±0.80	6.5 ^{ab} ±0.76	6.35 ^b ±1.08	6.42 ^b ±0.76
T3	5.43 ^c ±0.76	5.07 ^c ±0.73	5.00 ^c ±0.88	5.36 ^{bc} ±0.74	5.36 ^b ±0.93
T4	6.21 ^d ±0.89	5.35 ^c ±0.93	5.57 ^{bc} ±0.85	6.00 ^b ±0.78	6.14 ^b ±0.66
T5	4.71 ^c ±0.73	4.43 ^d ±0.76	4.86 ^c ±0.77	4.86 ^c ±0.77	4.71 ^c ±0.83
T6	5.14 ^c ±0.66	4.92 ^{dc} ±0.92	5.21 ^c ±0.89	5.36 ^{bc} ±0.84	5.35 ^b ±0.74
CD value	0.674	0.617	0.648	0.626	0.567

Note:

- C- Control sample.
- T1- Cakes prepared by incorporated 10% of whole rice bran.
- T2- Cakes prepared by incorporated 10% of stabilized whole rice bran.
- T3- Cakes prepared by incorporated 20% of whole rice bran.
- T4- Cakes prepared by incorporated 20% of stabilized whole rice bran.
- T5- Cakes prepared by incorporated 30% of whole rice bran.
- T6- Cakes prepared by incorporated 30% of stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

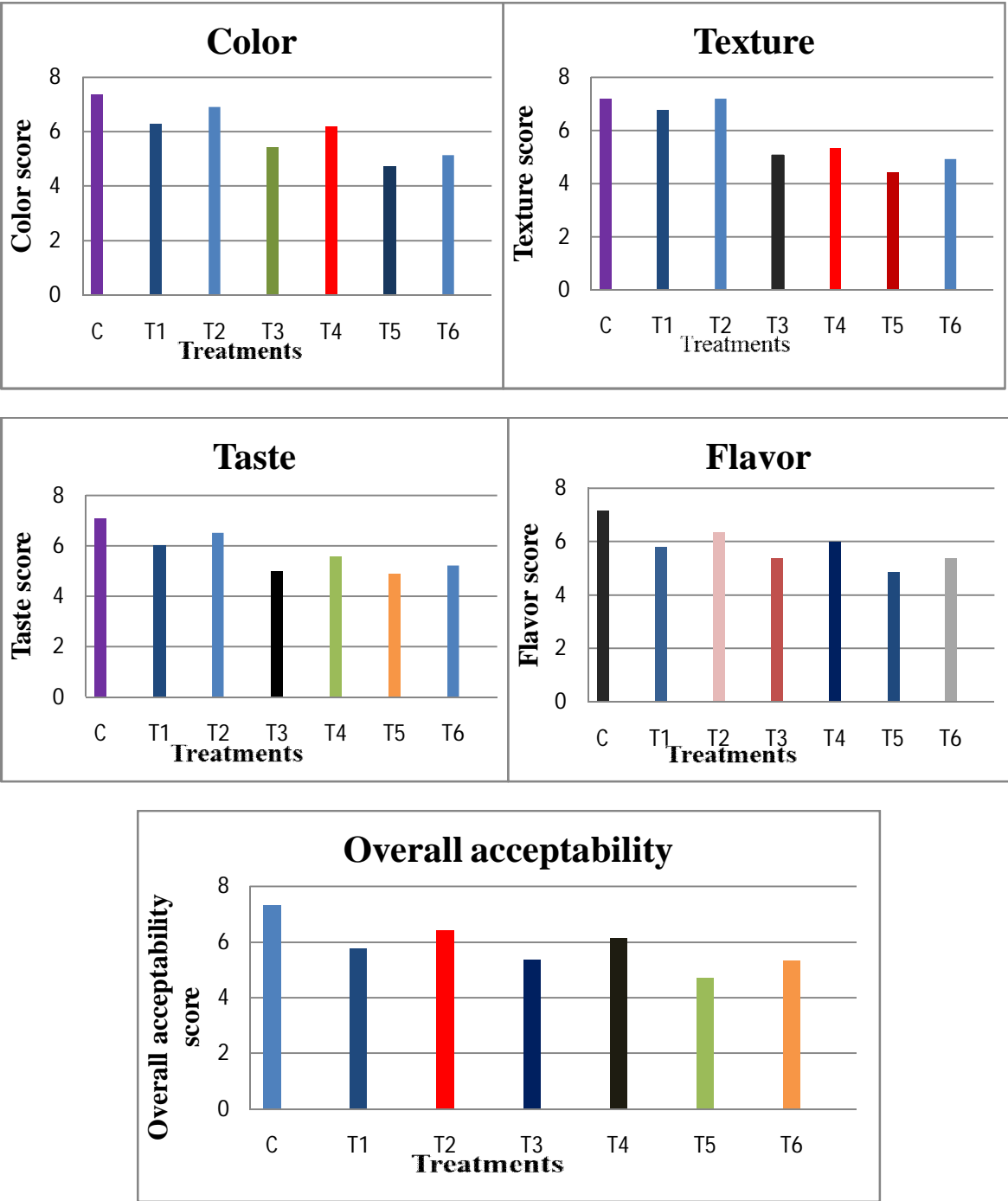


Fig 4.6 Mean sensory score of cakes

4.5 Physico-chemical and nutritional quality characteristics of baked products

4.5.1 Physical quality characteristics of baked products

4.5.1.1 Physical quality characteristics of biscuits

The result of the physical quality characteristics of the biscuits prepared with wheat and whole rice bran&stabilized whole rice bran flour incorporated biscuits is given in Table 4.7 and graphically as shown in Fig 4.7.

The diameter of the biscuits decreased from 41.07mm to 40.00mm with increase in the level of incorporation of WRB and SWRB. The results showed that diameter in control sample had the maximum diameter (41.07mm), followed by T1 (40.83mm), while minimum diameter was observed in T2 (40.00mm).

However, biscuit thickness increased from 7.88mm to 8.87mm with increase in the level of incorporation of WRB and SWRB. The results showed that T2 had maximum thickness (8.87mm) followed by T1 (8.61mm) while minimum thickness was observed in control sample (7.88mm).

The spread ratio was affected by the competition for the available water. The spread factor of biscuits decreased from 5.21 to 4.51 with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum spread factor (5.21), followed by T1 (4.74) while minimum spread factor was observed in T2 (4.51).

However, statistically significant difference was not observed in the diameter of the biscuits between the control and both the experimental samples (T1 and T2) and also between the experimental samples.

Statistically significant difference in the thickness and spread ratio of the biscuits between the control and both the experimental samples (T1 and T2) was observed.

Statistically significant difference in the thickness of the biscuits between the T1 and T2 was observed. Statistically significant difference was not observed in the spread ratio of biscuits between the T1 and T2.

Overall, it was found that the incorporation of rice bran at different levels had a significant effect on diameter, thickness and spread ratio of the biscuits.

Sharma and Chauhan (2002) reported that physical properties of biscuits like width, thickness, and spread ratio were affected significantly with the increase in the level of incorporation of bran and also by the method of stabilization.

Table 4.7: Physical quality characteristics of biscuits prepared by incorporating of rice bran

Treatments	Diameter (mm)	Thickness (mm)	Spread ratio (W/T)
C	41.07 ^a ±0.37	7.88 ^a ±0.08	5.21 ^a ±0.09
T1	40.83 ^a ±0.28	8.61 ^b ±0.03	4.74 ^b ±0.04
T2	40.00 ^a ±0.41	8.87 ^c ±0.08	4.51 ^b ±0.03
CD value	3.588	0.410	0.137

Note:

- C- Control sample.
- T1- Biscuit prepared by incorporated 10% of whole rice bran.
- T2- Biscuit prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

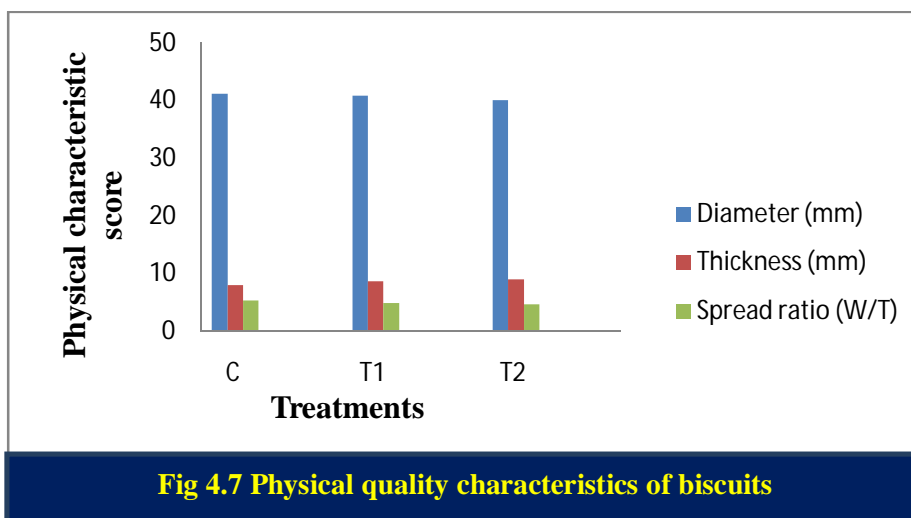


Fig 4.7 Physical quality characteristics of biscuits

4.5.1.2 Physical quality characteristics of cookies

The result of the physical quality characteristics of the cookies prepared with wheat and whole rice bran & stabilized whole rice bran flours incorporated cookies is given Table 4.8 and graphically as shown in Fig 4.8.

The diameter of the cookies decreased from 58.65mm to 54.04mm with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum diameter (58.65mm), followed by T1 (55.81mm) while minimum diameter was observed in T2 (54.04mm).

The cookies thickness increased from 15.60mm to 16.06mm with increase in the level of substitution of WRB and SWRB. The results showed that T2 had maximum thickness (16.06mm) followed by T1 (15.86mm) while minimum thickness was observed in control sample (15.60mm).

The spread ratio was affected by the competition for the available water. The spread factor of cookies decreased from 3.75 to 3.36 with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum spread factor (3.75), followed by T1 (3.51) while minimum spread factor was observed in T2 (3.36).

However, statistically significant difference was not observed in the diameter of the cookies between the control and both the experimental samples (T1 and T2) and also between T1 and T2.

Statistically significant difference was observed in the thickness of the cookies between the control and both the experimental samples (T1 and T2) and also between the T1 and T2.

Statistically significant difference in the spread factor of the cookies between the control and T2 was observed but statistically significant difference was not observed in the spread factor between the control and T1.

It was found that the incorporation of rice bran at different levels had a significant effect on diameter, thickness and spread ratio of the cookies.

Sudha *et al.*, (2007) showed that incorporation of bran at different levels resulted in a decrease in the spread factor of cookies without much change in thickness. Reduction of cookies diameter was also observed by Sievert *et al.*, (1990).

Table 4.8: Physical quality characteristics of cookies prepared by incorporating rice bran

Treatments	Diameter (mm)	Thickness (mm)	Spread ratio (W/T)
C	58.65 ^a ±0.06	15.60 ^a ±0.24	3.75 ^a ±0.01
T1	55.81 ^b ±0.03	15.86 ^b ±0.11	3.51 ^a ±0.03
T2	54.04 ^c ±0.06	16.06 ^b ±0.11	3.36 ^b ±0.03
CD value	0.108	0.060	0.271

Note:

- C- Control sample.
- T1- Cookies prepared by incorporated 10% of whole rice bran.
- T2- Cookies prepared by incorporated 20% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

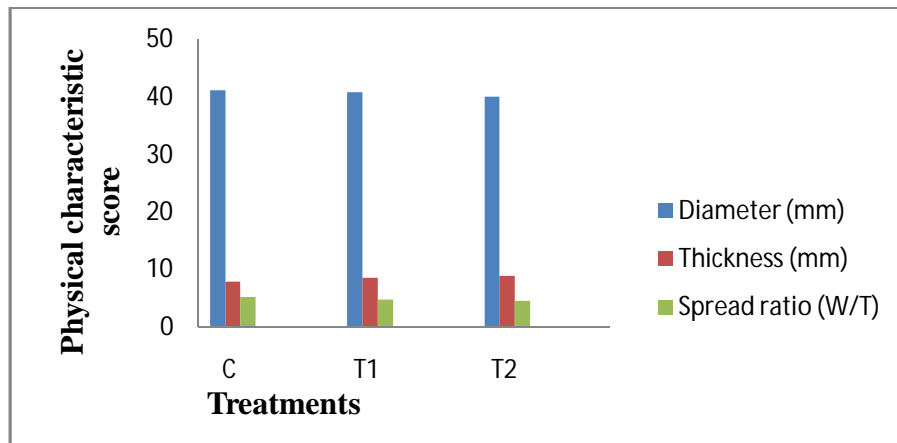


Fig 4.8 Physical quality characteristics of cookies

4.5.1.3 Physical quality characteristics of cakes

The result of the physical quality characteristics of the cakes prepared with wheat and whole rice bran & stabilized whole rice bran incorporated cookies is given in Table 4.9 and graphically as shown in Fig 4.9. It was showed observed that the incorporation of rice bran at different levels had a significant effect on height, length, width, volume and density of cakes.

The height of cakes decreased from 2.23cm to 1.93cm with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum height (2.23cm), followed by T2 (2.06cm) while minimum height was observed in T1 (1.93cm).

The length of cakes decreased from 15.00 cm to 14.16cm with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum length (15.00cm), followed by T2 (14.66cm) while minimum length was observed in T1 (14.16cm).

The width of the cake decreased from 4.50cm to 4.30cm with increase in the level of incorporation of rice bran. The results showed that C and T2 had maximum width (4.50cm) while minimum width was observed in T1 (4.30cm).

The volume of the cake decreased from 150.52cm to 111.51cm with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had maximum volume (150.52cm) followed by T2 (135.89cm) while minimum volume was observed in T1 (111.51cm).

The density of cakes decreased from 1.14kg/cm³ to 1.06 kg/cm³ with increase in the level of incorporation of WRB and SWRB. The results showed that control sample had the maximum density (1.14kg/cm³), followed by T1 (1.08 kg/cm³) while minimum density was observed in T2 (1.08 kg/cm³).

However, statistically significant difference in the height, length, width and volume of the cakes between the control and both the experimental samples (T1 and T2) was observed but there was a non-significant difference between the T1 and T2.

Statistically significant difference in the density values between the rice bran incorporated cakes and control was observed.

Table 4.9: Physical quality characteristics of cakes prepared by incorporating rice bran

Treatments	Height (cm)	Length (cm)	Width (cm)	Volume (cm)	Density (kg/cm³)
C	2.23 ^a ±0.12	15.00 ^a ±0.00	4.50 ^a ±0.00	150.52	1.14 ^a ±0.02
T1	1.93 ^b ±0.06	14.16 ^b ±0.29	4.30 ^b ±0.00	117.51	1.08 ^b ±0.01
T2	2.06 ^{ab} ±0.12	14.66 ^a ±0.29	4.50 ^a ±0.00	135.89	1.06 ^b ±0.01
CD value	0.171	0.384	0.00	0.00	0.027

Note:

- C- Control sample.
- T1- Cakes prepared by incorporated 10% of whole rice bran.
- T2- Cakes prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

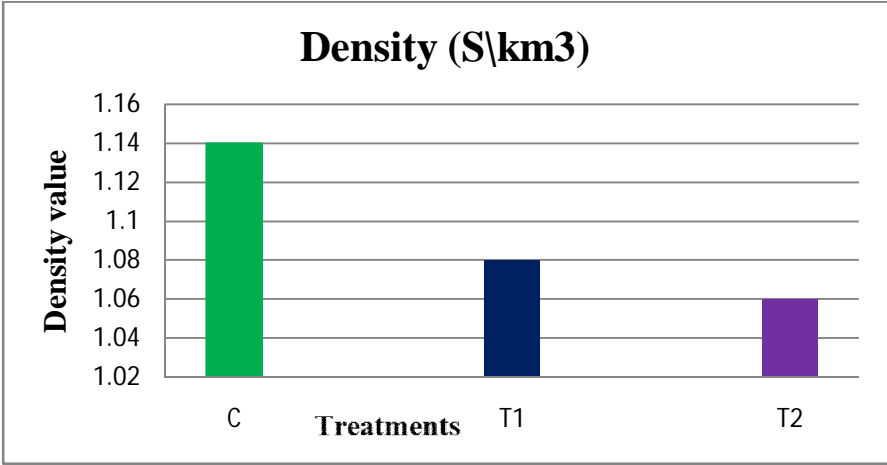
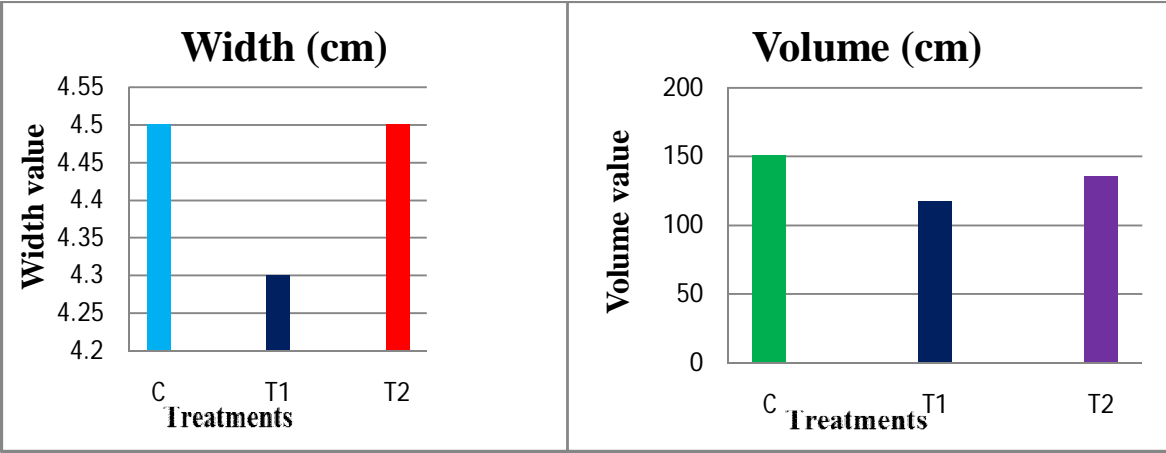
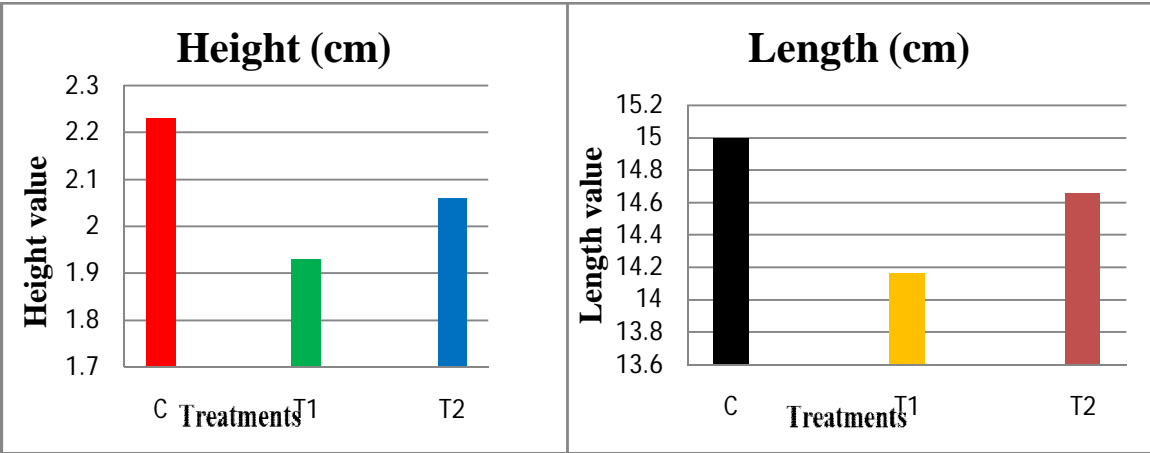


Fig 4.9 Physical quality characteristics of cakes

4.5.2 Chemical and nutritional quality characteristics of baked products

4.5.2.1 Chemical and nutritional quality characteristics of biscuits

The results of chemical and nutritional quality characteristics of biscuits prepared with wheat flour with and without the addition of rice bran is tabulated in Table 4.10 and graphically as shown in Fig 4.10.

4.5.2.1.a Moisture

The moisture content was $3.22\pm 0.02\%$, $2.89\pm 0.02\%$ and $2.82\pm 0.04\%$ for the control, T1 and T2 respectively.

The moisture content of control sample was significantly higher ($P<0.05$) in the control sample ($3.22\pm 0.02\%$) compared to T1 and T2. The moisture content of T1 was significantly higher ($P<0.05$) compared to T2.

However, statistically significant difference was observed between the control and both the experimental samples (T1 and T2). Statistically significant difference was not observed between the T1 and T2.

4.5.2.1.b Protein

The protein content was $5.28\pm 0.04\%$, $6.50\pm 0.02\%$ and $6.54\pm 0.02\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the protein content of control and both the experimental samples (T1 and T2) but there is no significant difference in the protein content of T1 and T2.

The protein content of biscuits increased from 5.28% to 6.54% with increase in the level of incorporation of WRB and SWRB. Similar result also reported by Banureka and Mahendran(2009) in the formulation of wheat-soybean biscuits.

4.5.2.1.c Ash

The ash content was $0.70\pm 0.20\%$, $1.26\pm 0.04\%$ and $1.04\pm 0.07\%$ for the control, T1 and T2 respectively.

The ash content of biscuits increased from 0.70% to 1.26% with increase in the level of incorporation of WRB and SWRB. The results showed that T1 had the maximum

ash content (1.26%), followed by T2 (1.04%) while minimum ash content was observed in control sample *i.e.* C (0.70%).

Statistically significant difference was observed in ash content between the control and both the experimental samples (T1 and T2) but there is no significant difference in ash content between the T1 and T2.

4.5.2.1.d Crude fibre

The crude fibre content was $0.59\pm 0.12\%$, $2.38\pm 0.05\%$ and $2.07\pm 0.10\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the crude fibre content of control and both the experimental samples (T1 and T2) and also between the treatment of T1 and T2.

The crude fibre content of biscuits increased from 0.59% to 2.38% with increase in the level of substitution of WRB and SWRB.

4.5.2.1.e Fat

The fat content was $18.56\pm 0.07\%$, $22.74\pm 0.21\%$ and $22.66\pm 0.08\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the fat content of control and both the experimental samples (T1 and T2) but there is no significant difference in fat content of biscuits within the treatment.

4.5.2.1.f Carbohydrate

The carbohydrate content was $56.28\pm 0.04\%$, $55.82\pm 0.08\%$ and $55.24\pm 0.48\%$ for the control, T1 and T2 respectively.

The carbohydrate content of biscuits decreased from 56.28% to 50.26% with increase in the level of substitution of WRB and SWRB. The results showed that the control had the maximum carbohydrate content (56.28%) and T2 had the lowest carbohydrate content (55.24%)

Statistically significant difference was not observed in the carbohydrate content of control and both the experimental samples (T1 and T2) and also between the treatment of T1 and T2.

Table 4.10: Chemical and nutritional quality characteristics of biscuits

Nutrients	C	T1	T2	CD value
Moisture (%)	3.22 ^a ±0.02	2.89 ^b ±0.03	2.82 ^b ±0.04	0.076
Protein (%)	5.28 ^a ±0.04	6.50 ^b ±0.02	6.54 ^b ±0.02	0.052
Ash (%)	0.70 ^a ±0.20	1.26 ^b ±0.04	1.04 ^c ±0.07	0.207
Crude fiber (%)	0.59 ^a ±0.12	2.38 ^b ±0.05	2.07 ^c ±0.10	0.181
Fat (%)	18.56 ^a ±0.07	22.74 ^b ±0.21	22.66 ^b ±0.08	0.277
Carbohydrate (%)	56.28 ^a ±0.04	55.82 ^a ±0.08	55.24 ^a ±0.48	0.583

Note:

- C- Control sample.
- T1- Biscuits prepared by incorporated 10% of whole rice bran.
- T2- Biscuits prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

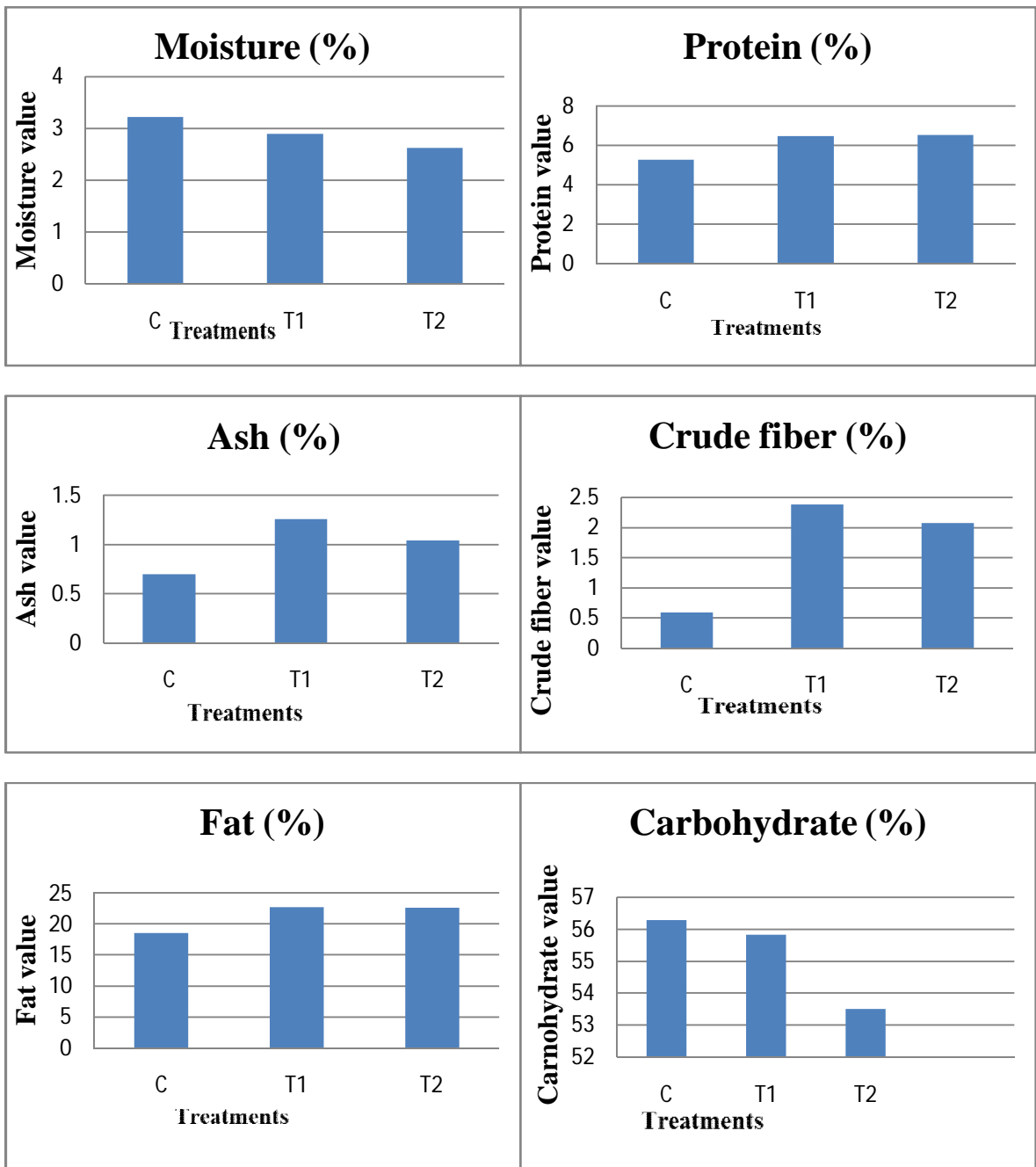


Fig 4.10 Nutritional quality characteristics of biscuits

4.5.2.2 Chemical and nutritional quality characteristics of cookies

The results of chemical and nutritional quality characteristics of cookies prepared with wheat flour with and without the addition of rice bran is tabulated in Table 4.11 and graphically as shown in Fig 4.11.

The moisture content was $5.69\pm 0.03\%$, $5.47\pm 0.02\%$ and $4.99\pm 0.10\%$ for the control, T1 and T2 respectively.

The moisture content of cookies decreased from 5.69% to 4.99% with increase in the level of incorporation of WRB and SWRB. The results showed that control had the maximum moisture content (5.69%), followed by T1 (5.47%) while minimum moisture content of cookies was observed in T2 (4.99%).

The protein content was $5.39\pm 0.12\%$, $5.58\pm 0.06\%$ and $6.15\pm 0.09\%$ for the control, T1 and T2 respectively.

The protein content of cookies increased from 5.39% to 6.15% with increase in the level of incorporation of WRB and SWRB. The results showed that T2 had the maximum protein content (6.15%), followed by T1 (5.58%) while minimum protein was observed in C (5.39%).

The ash content was $1.75\pm 0.07\%$, $1.96\pm 0.07\%$ and $2.19\pm 0.06\%$ for the control, T1 and T2 respectively.

The ash content of cookies increased from 1.75% to 2.19% with increase in the level of incorporation of WRB and SWRB. The results showed that T2 had the maximum ash content (2.19%), followed by T1 (1.96%) while minimum ash was observed in C (1.75%).

The crude fibre content was $1.89\pm 0.06\%$, $2.12\pm 0.04\%$ and $2.34\pm 0.09\%$ for the control, T1 and T2 respectively.

The crude fibre content of cookies increased from 1.89% to 2.34% with increase in the level of incorporation of WRB and SWRB. The results showed that T2 had the maximum fibre content (2.34%), followed by T1 (2.12%) while minimum fibre was observed in C (1.89%).

The fat content was $19.08\pm 0.17\%$, $23.48\pm 0.16\%$ and $24.39\pm 0.22\%$ for the control, T1 and T2 respectively.

The fat content of cookies increased from 19.08% to 24.39% with increase in the level of incorporation of WRB and SWRB. The results showed that T2 has the maximum fat content (24.39%), followed by T1 (23.48%) while minimum fat was observed in C (19.08%).

The carbohydrate content was $64.75 \pm 0.07\%$, $64.17 \pm 0.19\%$ and $63.40 \pm 0.08\%$ for the control, T1 and T2 respectively.

The carbohydrate content of cookies decreased from 64.75% to 59.61% with increase in the level of incorporation of rice bran. The results showed that control sample has the maximum carbohydrate content (64.75%), followed by T1 (64.17%) while minimum carbohydrate was observed in T2 (63.40%)

However, statistically significant difference was observed in the moisture, ash, protein, fat, carbohydrate and crude fibre between the control and both the experimental samples (T1 and T2) and also between the treatment of T1 and T2.

Carver (2006) reported that the hardness of cookies increased in different levels of incorporation of rice bran and this was mainly due to the extent of protein-starch interaction in the flour.

Table 4.11: Chemical and nutritional quality characteristics of cookies

Nutrients	C	T1	T2	CD value
Moisture (%)	5.69 ^a ±0.03	5.47 ^b ±0.04	4.99 ^c ±0.10	0.122
Protein (%)	5.39 ^a ±0.12	5.58 ^b ±0.06	6.15 ^c ±0.09	0.157
Ash (%)	1.75 ^a ±0.07	1.96 ^b ±0.07	2.19 ^c ±0.06	0.113
Crude fiber (%)	1.89 ^a ±0.06	2.12 ^b ±0.04	2.34 ^c ±0.09	0.113
Fat (%)	19.08 ^a ±0.17	23.48 ^b ±0.16	24.39 ^c ±0.22	0.306
Carbohydrate (%)	64.75 ^a ±0.07	64.17 ^b ±0.19	63.40 ^c ±0.08	0.400

Note:

- C- Control sample.
- T1- Cookies prepared by incorporated 10% of whole rice bran.
- T2- Cookies prepared by incorporated 20% of stabilized whole rice bran.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

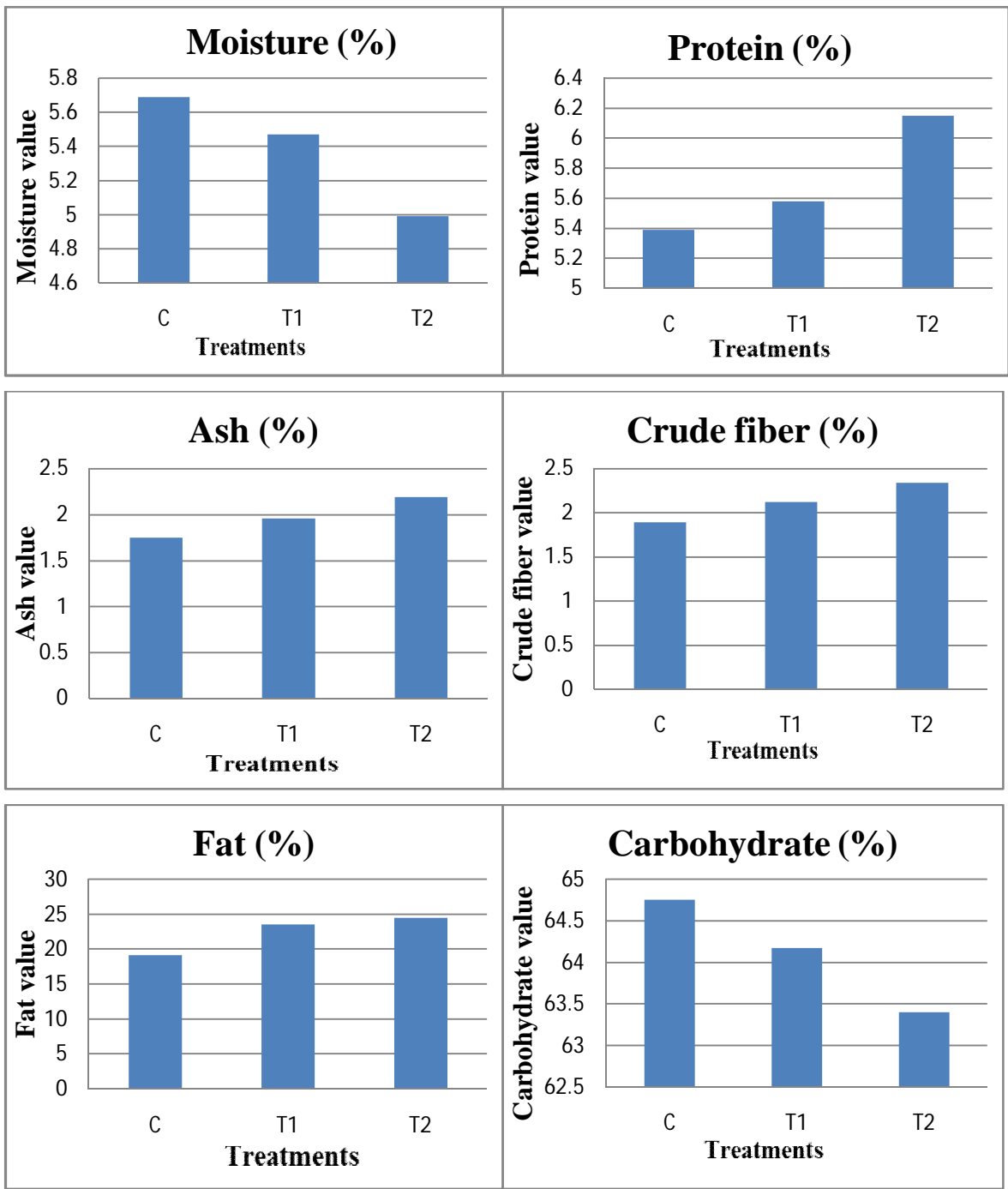


Fig 4.11 Nutritional quality characteristics of cookies

4.5.2.3 Chemical and nutritional quality characteristics of cakes

The results of chemical and nutritional characteristics of cakes prepared with wheat flour with and without the addition of rice bran are tabulated in Table 4.12 and graphically as shown in Fig 4.12.

4.5.2.3.a Moisture

The moisture content was $14.80\pm 0.37\%$, $14.05\pm 0.08\%$ and $13.86\pm 0.14\%$ for the control, T1 and T2 respectively.

The moisture content of control sample was significantly higher ($P<0.05$) in the control sample ($14.80\pm 0.37\%$) compared to T1 and T2.

However, statistically significant difference was observed between the control and both the experimental samples (T1 and T2) but there is no significant difference in the moisture content of cakes within the treatment.

4.5.2.3.b Protein

The protein content was $7.88\pm 0.19\%$, $8.46\pm 0.22\%$ and $8.68\pm 0.08\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the protein content of cakes between the control and both the experimental samples (T1 and T2) but there is no significant difference in protein content of cakes within the treatment.

4.5.2.3.c Ash

The ash content was $0.75\pm 0.07\%$, $1.59\pm 0.12\%$ and $1.34\pm 0.13\%$ for the control, T1 and T2 respectively.

The ash content of cakes increased from 0.75% to 1.59% with increase in the level of incorporation of WRB and SWRB. The results showed that T1 had the maximum ash content (1.59%), followed by T2(1.34%) while minimum ash content was observed in control *i.e.* (0.75%).

Statistically significant difference was observed in ash content cakes between the control and both the experimental samples (T1 and T2) but there is no significant difference in ash content of cakes within the treatment.

4.5.2.3.d Crude fibre

The crude fibre content was $0.88\pm 0.07\%$, $1.24\pm 0.10\%$ and $1.13\pm 0.10\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the fibre content of cakes between the control and both the experimental samples (T1 and T2) but there is no significant difference in crude fibre content of cakes within the treatment.

The crude fibre content of cakes increased from 0.88% to 1.24% with increase in the level of incorporation of whole rice bran and stabilized whole rice bran.

4.5.2.3.e Fat

The fat content was $19.35\pm 0.07\%$, $22.83\pm 0.09\%$ and $22.69\pm 0.16\%$ for the control, T1 and T2 respectively.

Statistically significant difference was observed in the fat content of cakes between the control and both the experimental samples (T1 and T2) but there is no significant difference in fat content of cakes within the treatment.

4.5.2.3.f Carbohydrate

The carbohydrate content was $50.74\pm 0.07\%$, $50.27\pm 0.05\%$ and $49.53\pm 0.89\%$ for the control, T1 and T2 respectively.

The carbohydrate content of cakes decreased from 50.74% to 49.53% with increase in the level of incorporation of WRB and SWRB. The results showed that the control had the maximum carbohydrate content (50.74%) and T2 has the lowest carbohydrate content (49.53%)

Statistically significant difference was observed in the carbohydrate content of cakes between the control and both the experimental samples (T1 and T2).

The decrease in carbohydrate content was due to the fact that cake flour was main contributor to the carbohydrate. Similar results were observed by Rascoet *al.*, (1989) in the baked products made by the incorporating the different level of germinated grains.

Table 4.12: Chemical and nutritional quality characteristics of cakes

Nutrients	C	T1	T2	CD value
Moisture (%)	14.80 ^a ±0.37	14.05 ^b ±0.08	13.86 ^b ±0.14	0.389
Protein (%)	7.88 ^a ±0.19	8.46 ^b ±0.22	8.68 ^b ±0.08	0.291
Ash (%)	0.75 ^a ±0.07	1.59 ^b ±0.13	1.34 ^b ±0.12	0.249
Crude fiber (%)	0.88 ^a ±0.07	1.24 ^b ±0.10	1.13 ^b ±0.04	0.122
Fat (%)	19.35 ^a ±0.07	22.83 ^b ±0.09	22.69 ^b ±0.16	0.208
Carbohydrate (%)	50.74 ^a ±0.07	50.27 ^b ±0.05	49.53 ^b ±0.89	0.914

Note:

- C- Control sample.
- T1- Cakes prepared by incorporated 10% of whole rice bran.
- T2- Cakes prepared by incorporated 10% of whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

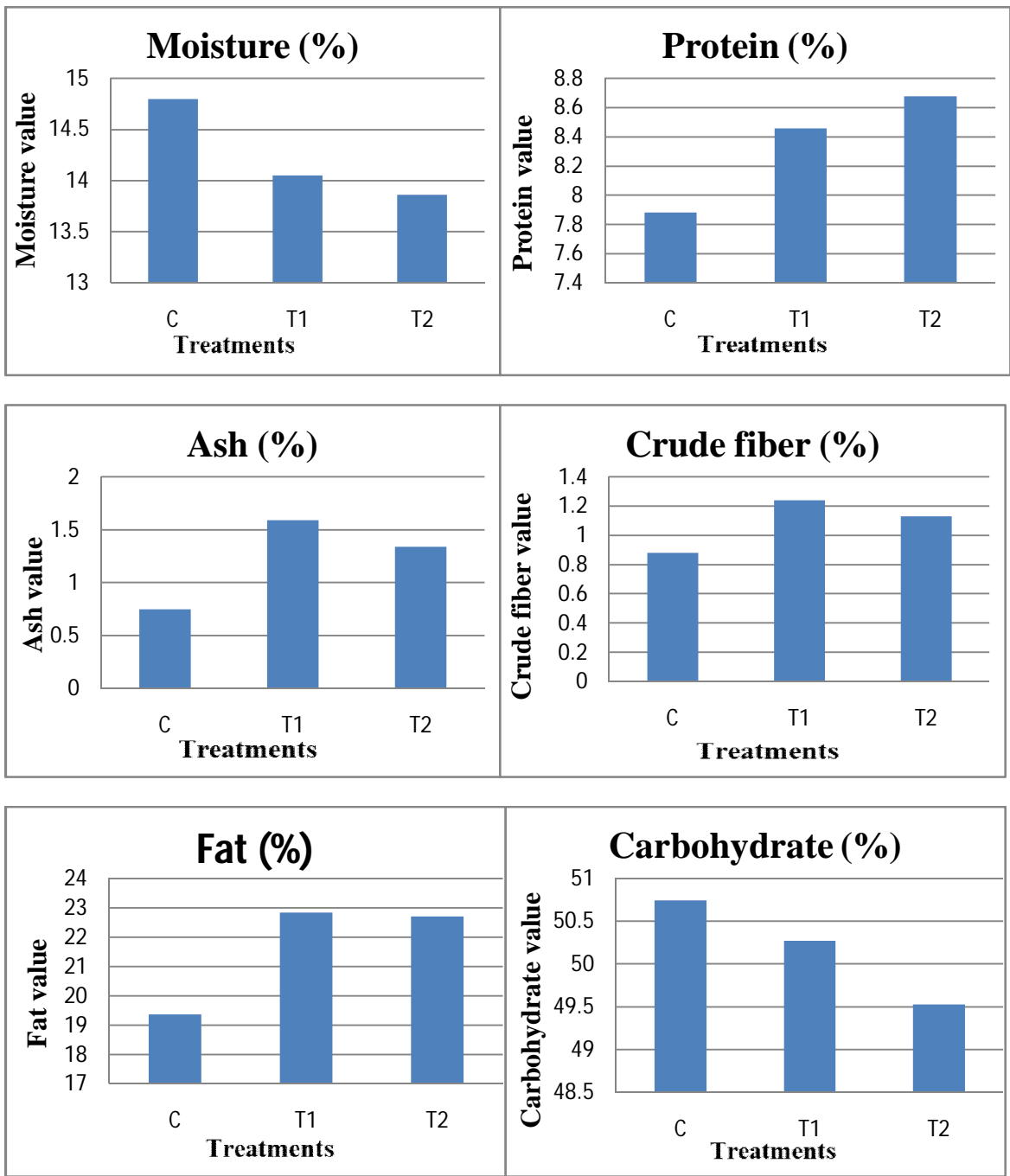


Fig 4.12 Nutritional quality characteristics of cakes

4.6 Shelf life studies of baked products

4.6.1 Mean sensory scores of biscuits on storage

Biscuits (control, T1 and T2) were kept for storage study. The samples were observed daily for visual difference and were subjected to sensory evaluation on 0th, 15th and 30th day.

The results of the mean sensory evaluation of biscuits from initial day to end of storage period are presented in the Table 4.13.

The changes in mean sensory scores of biscuits decreased from initial to 30th day of storage period in both whole rice bran and stabilized whole rice bran incorporated biscuits.

The sensory parameters in control sample were decreased from initial to 30th day for color (7.93 to 6.69), texture (7.50 to 6.62), taste (7.50 to 6.85), flavor (7.50 to 6.23) and overall acceptability (7.93 to 6.69) respectively. In the same way the mean sensory scores decreased significantly from initial to 30th day in whole rice bran and stabilized whole rice bran incorporated biscuits.

In 10% whole rice bran incorporated biscuits the sensory score decreased from 6.86 to 6.00 for color, 6.86 to 5.54 for texture, 6.29 to 6.08 for taste, 6.43 to 5.85 for flavor and 6.36 to 6.08 for overall acceptability. In 10% stabilized whole rice bran incorporated biscuits the sensory score decreased from 7.07 to 6.31 for color, 7.21 to 5.92 for texture, 6.71 to 6.46 for taste, 6.71 to 6.00 for flavor and 6.79 to 6.54 for overall acceptability.

Statistical analysis revealed a significant difference for all the sensory characteristics of biscuits between the treatments and both before and after storage period of 0th, 15th and 30th day.

The declining trend in quality scores for texture was due to absorption of moisture from the atmosphere that has inverse correlation with texture in biscuits reported by Sharif *et al.* (2005).

Shelf life is a major consideration in developing, producing and marketing of food product, it refers to the time during which a product remains 'acceptable' to a consumer in terms of sensory characters. Many factors influence the shelf life of the product like, moisture loss, spoilage due to microorganisms, enzymatic changes and oxidation reported by Adegoke *et al.* (1998).

Table 4.13: Mean sensory scores of biscuits on storage

Treatments	Duration (Days)	Sensory attributes				
		Color	Texture	Taste	Flavor	Overall acceptability
C	0 th day	7.93 ^a ±0.83	7.50 ^a ±0.85	7.50 ^a ±0.94	7.50 ^a ±0.85	7.93 ^a ±0.73
	15 th day	7.42 ^a ±0.79	7.00 ^a ±0.74	7.17 ^a ±0.72	7.42 ^a ±0.79	7.33 ^a ±0.49
	30 th day	6.69 ^a ±0.75	6.62 ^a ±0.65	6.85 ^a ±0.69	6.23 ^a ±0.60	6.69 ^a ±0.63
T1	0 th day	6.86 ^b ±0.77	6.86 ^a ±0.86	6.29 ^b ±0.91	6.43 ^b ±0.85	6.36 ^b ±0.63
	15 th day	6.50 ^b ±0.90	6.67 ^a ±1.07	6.17 ^b ±0.72	6.43 ^b ±0.79	6.67 ^b ±0.65
	30 th day	6.00 ^b ±0.71	5.54 ^b ±0.88	6.08 ^a ±0.86	5.85 ^a ±0.99	6.08 ^{bc} ±0.64
T2	0 th day	7.07 ^b ±0.83	7.21 ^a ±0.58	6.71 ^b ±0.91	6.71 ^b ±1.07	6.79 ^b ±0.80
	15 th day	7.00 ^{ab} ±0.85	6.75 ^a ±0.75	6.58 ^{ab} ±0.67	6.50 ^b ±1.17	6.75 ^b ±0.75
	30 th day	6.31 ^b ±0.63	5.92 ^b ±0.76	6.46 ^{ab} ±0.52	6.00 ^a ±1.00	6.54 ^{ab} ±0.88
CD Value	0 th day	0.596	0.664	0.627	0.637	0.543
	15 th day	0.658	0.742	0.585	0.720	0.533
	30 th day	0.562	0.643	0.552	0.666	0.554

Note:

- C- Control sample.
- T1- Biscuits prepared by incorporated 10% of whole rice bran.
- T2- Biscuits prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.5.2 Nutritional characteristics of biscuits during storage

The results of analysis of chemical and nutritional quality characteristics of biscuits during storage are shown in Table 4.14.

The moisture content of biscuits before storage was 3.22%, 2.89% and 2.82% for the control, T1 and T2 respectively. The moisture content of biscuit after storage was 3.28%, 3.09% and 2.98% for the control, T1 and T2 respectively.

The results revealed that there was a statistically significant increase in the mean moisture content of biscuits was observed from 0th day to 30th day of storage period. The increase in moisture content of biscuits might be due to hygroscopic nature of rice bran and wheat flour. Similar results were reported by Kumar and Barmanray (2007) in the button mushroom powder fortified biscuits.

The protein content of biscuits before storage was 5.28%, 6.50% and 6.54% for the control, T1 and T2 respectively. The protein content of biscuits after storage was 5.21%, 6.42% and 6.20% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in mean protein content of biscuits from 0th day to 30th day of storage period as shown in the Table 4.14. The decrease in protein content during storage might be due to hydrolysis of peptide bonds by the help of protease enzyme that cause splitting of protein molecules during storage. Similar decrease of protein content with storage period in single cell protein biscuits was reported by Kanchana *et al.*, (2008).

The ash content of biscuits before storage was 0.70%, 1.26% and 1.04% for the control, T1 and T2 respectively. The ash content of biscuits after storage was 0.63%, 1.05% and 0.89% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in the mean ash content of biscuits from 0th day to 30th days of storage period as shown in the Table 4.14.

The crude fibre content of biscuits before storage was 0.59%, 2.38% and 2.07% for the control, T1 and T2 respectively. The crude fibre content of biscuits after storage was 0.52%, 2.26% and 1.87% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in mean crude fibre content of biscuits from 0th day to 30th day of storage period as shown in the Table 4.14. The decrease in crude fiber might be due to the degradation of hemicelluloses and other

structural polysaccharides during storage and also heat and moisture solubilizers degrade pectic substances leading to the decrease in the fibre content of the biscuits was reported by Sharon and Usha, (2006).

The fat content of biscuits before storage was 18.56%, 22.74% and 22.66% for the control, T1 and T2 respectively. The fat content of biscuits after storage was 18.35%, 22.66% and 22.61% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in mean fat content of biscuits from 0th day to 30th day of storage period as showed in the Table 4.14. This decrease in fat content during storage was due to the incorporation of moisture in biscuits from the atmosphere and due to oxidation of fatty acids resulting in free fatty acid formation.

The carbohydrate content of biscuits before storage was 56.28%, 55.82% and 55.24% for the control, T1 and T2 respectively. The carbohydrate content of biscuits after storage was 56.14%, 55.48% and 54.84% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in mean carbohydrate content of biscuits from 0th day to 30th day of storage period as showed in the Table 4.14.

Table 4.14: Nutritional characteristics of biscuits during storage

Nutrients	Duration	C	T1	T2	CD value
Moisture (%)	Before	3.22 ^a ±0.02	2.89 ^b ±0.03	2.82 ^b ±0.04	0.076
	After	3.28 ^a ±0.04	3.09 ^b ±0.04	2.98 ^c ±0.08	0.108
Protein (%)	Before	5.28 ^a ±0.04	6.50 ^b ±0.02	6.54 ^b ±0.02	0.052
	After	5.21 ^a ±0.04	6.42 ^b ±0.05	6.20 ^c ±0.14	0.201
Ash (%)	Before	0.70 ^a ±0.20	1.26 ^b ±0.04	1.04 ^c ±0.07	0.207
	After	0.63 ^a ±0.06	1.05 ^b ±0.06	0.89 ^c ±0.03	0.089
Crude fiber (%)	Before	0.59 ^a ±0.12	2.38 ^b ±0.05	2.07 ^c ±0.10	0.181
	After	0.52 ^a ±0.14	2.26 ^b ±0.16	1.87 ^c ±0.06	0.224
Fat (%)	Before	18.56 ^a ±0.07	22.74 ^b ±0.21	22.66 ^{bc} ±0.08	0.277
	After	18.35 ^a ±0.07	22.66 ^b ±0.22	22.61 ^b ±0.11	0.406
Carbohydrate (%)	Before	56.28 ^a ±0.04	55.82 ^a ±0.08	55.24 ^a ±0.48	0.583
	After	56.14 ^c ±0.38	55.48 ^b ±0.61	54.84 ^b ±0.18	0.708

Note:

- C- Control sample.
- T1- Biscuits prepared by incorporated 10% of whole rice bran.
- T2- Biscuits prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.6.3 Mean sensory scores of cookies on storage

Cookies (Control, T1 and T2) were kept for storage study. The samples were observed daily for visual difference and were subjected to sensory evaluation on 0th, 15th and 30th day.

The results of the mean sensory evaluation of cookies from initial day to end of storage period are presented in the Table 4.15.

The changes in mean sensory scores of cookies decreased from initial to 30th day of storage period in both whole rice bran and stabilized whole rice bran incorporated cookies.

The sensory parameters in control sample were decreased from initial to 30th day for color (8.00 to 6.70), texture (7.35 to 6.50), taste (7.59 to 7.00), flavor (7.53 to 6.80) and overall acceptability (8.0 to 6.80) respectively. In the same way the mean sensory scores decreased significantly from initial to 30th day in whole rice bran and stabilized whole rice bran incorporated cookies.

In 10% whole rice bran incorporated cookies the sensory score decreased from 6.35 to 5.60 for color, 6.82 to 5.70 for texture, 6.12 to 5.70 for taste, 6.00 to 5.40 for flavor and 6.41 to 5.10 for overall acceptability. In 20% stabilized whole rice bran incorporated cookies the sensory score decreased from 7.06 to 6.30 for color, 7.18 to 6.30 for texture, 6.94 to 6.40 for taste, 6.71 to 6.50 for flavor and 6.71 to 6.40 for overall acceptability.

Statistical analysis revealed a significant difference for all the sensory characteristics of cookies between the treatments and both before and after storage period of 0th, 15th and 30th days.

Table 4.15: Mean sensory scores of cookies on storage

Treatment	Duration (Days)	Sensory attributes				
		Color	Texture	Taste	Flavor	Overall acceptability
T0	0 th day	8.00 ^a ±0.87	7.35 ^a ±0.79	7.59 ^a ±0.94	7.53 ^a ±0.87	8.00 ^a ±0.79
	15 th day	7.38 ^a ±0.77	7.08 ^a ±0.76	7.15 ^a ±0.69	7.38 ^a ±0.77	7.31 ^b ±0.48
	30 th day	6.70 ^a ±0.8	6.50 ^a ±0.85	7.00 ^a ±0.67	6.80 ^a ±0.63	6.80 ^a ±0.63
T1	0 th day	6.35 ^b ±0.79	6.82 ^a ±1.07	6.12 ^b ±1.11	6.00 ^b ±0.87	6.41 ^b ±0.69
	15 th day	6.26 ^b ±0.88	6.62 ^a ±1.04	6.08 ^b ±0.76	5.88 ^b ±0.77	5.76 ^b ±0.65
	30 th day	5.60 ^b ±0.52	5.70 ^b ±0.67	5.70 ^b ±0.82	5.40 ^b ±0.70	5.10 ^b ±0.57
T2	0 th day	7.06 ^{ab} ±0.75	7.18 ^b ±0.53	6.94 ^c ±0.90	6.71 ^b ±1.10	6.71 ^b ±0.77
	15 th day	6.92 ^b ±0.86	6.77 ^a ±0.73	6.54 ^{bc} ±0.66	6.46 ^b ±1.13	6.69 ^b ±0.75
	30 th day	6.30 ^b ±0.67	6.30 ^b ±0.82	6.40 ^{ac} ±0.52	6.50 ^a ±0.71	6.40 ^b ±0.84
CD Value	0 th day	0.596	0.664	0.627	0.637	0.543
	15 th day	0.618	0.701	0.566	0.666	0.503
	30 th day	0.615	0.726	0.621	0.643	0.591

Note:

- C- Control sample.
- T1- Cookies prepared by incorporated 10% of whole rice bran.
- T2- Cookies prepared by incorporated 20% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.6.4 Nutritional quality characteristics of cookies during storage

The moisture content of cookies before storage was 5.69%, 5.47% and 4.99% for the control, T1 and T2 respectively. The moisture content of cookies after storage was 5.73%, 5.57% and 5.06% for the control, T1 and T2 respectively.

The results revealed that there was significant increase in mean moisture content of cookies from 0th day to 30th day of storage period. The increase in moisture content of cookies might be due to hygroscopic nature of rice bran and wheat flour.

The protein content of cookies before storage was 5.39%, 5.58% and 6.15% for the control, T1 and T2 respectively. The protein content of cookies after storage was 5.28%, 5.41% and 5.72% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in the mean protein content of cookies from 0th day to 30th day of storage as showed in the Table 4.16. The decrease in protein content during storage might be due to hydrolysis of peptide bonds by the help of protease enzyme that cause splitting of protein molecules during storage was reported by Pasha *et al.*, (2002) in cookies supplemented with fiber from different sources.

The ash content of cookies before storage was 1.75%, 1.96% and 2.19% for the control, T1 and T2 respectively. The ash content of cookies after storage was 1.41%, 1.61% and 1.79% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in the mean ash content of cookies from 0th day to 30th day of storage as showed in the Table 4.16.

The crude fibre content of cookies before storage was 1.89%, 2.12% and 2.34% for the control, T1 and T2 respectively. The crude fibre content of cookies after storage was 1.77%, 2.02% and 2.16% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in the mean crude fibre content of cookies from 0th day to 30th day of storage as showed in the Table 4.16. The decrease in crude fiber might be due to the degradation of hemicelluloses and other structural polysaccharides during storage.

The fat content of cookies before storage was 19.08%, 23.48% and 24.39% for the control, T1 and T2 respectively. The fat content of cookies after storage was 19.05%, 23.42% and 24.37% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in the mean fat content of cookies from 0th day to 30th day of storage period as showed in the Table 4.16. This decreased in fat content during storage was due to the incorporation of moisture in cookies from the atmosphere and due to oxidation of fatty acids resulting in free fatty acid formation.

The carbohydrate content of cookies before storage was 64.75%, 64.17% and 63.40% for the control, T1 and T2 respectively. The carbohydrate content of cookies after storage was 64.68%, 64.00% and 63.32% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in mean carbohydrate content of cookies from 0th day to 30th day of storage period as showed in the table 4.16.

Table 4.16: Nutritional characteristics of cookies during storage

Nutrients	Duration	C	T1	T2	CD value
Moisture (%)	Before	5.69 ^a ±0.03	5.47 ^b ±0.04	4.99 ^c ±0.10	0.122
	After	5.73 ^a ±0.03	5.57 ^b ±0.05	5.06 ^c ±0.05	0.090
Protein (%)	Before	5.39 ^a ±0.12	5.58 ^b ±0.06	6.15 ^c ±0.09	0.157
	After	5.28 ^a ±0.02	5.41 ^b ±0.04	5.72 ^c ±0.06	0.134
Ash (%)	Before	1.75 ^a ±0.07	1.96 ^b ±0.07	2.19 ^c ±0.06	0.113
	After	1.41 ^a ±0.05	1.61 ^b ±0.06	1.79 ^c ±0.03	0.091
Crude fiber (%)	Before	1.89 ^a ±0.06	2.12 ^b ±0.04	2.34 ^c ±0.09	0.113
	After	1.77 ^a ±0.04	2.02 ^b ±0.06	2.16 ^c ±0.05	0.087
Fat (%)	Before	19.08 ^a ±0.17	23.48 ^b ±0.16	24.39 ^c ±0.22	0.306
	After	19.05 ^a ±0.23	23.42 ^b ±0.16	24.37 ^c ±0.19	0.317
Carbohydrate (%)	Before	64.75 ^a ±0.07	64.17 ^b ±0.19	63.40 ^c ±0.08	0.400
	After	64.68 ^c ±0.42	64.00 ^b ±0.04	63.32 ^b ±0.60	0.685

Note:

- C- Control sample.
- T1- Cookies prepared by incorporated 10% of whole rice bran.
- T2- Cookies prepared by incorporated 20% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.6.5 Mean sensory scores of cakes on storage

Cakes (Control, T1 and T2) were kept for storage study. The samples were observed daily for visual difference and were subjected to sensory evaluation on 0th, 3rd, 6th day.

The results of the mean sensory evaluation of cakes from initial day to end of storage period are presented in the Table 4.17.

The changes in mean sensory scores of cakes decreased from initial to 6th day of storage period in both whole rice bran and stabilized whole rice bran incorporated cakes.

The sensory parameters in control sample were decreased from initial to 6th day for color (7.36 to 6.92), texture (7.21 to 6.31), taste (7.07 to 6.54), flavor (7.14 to 6.08) and overall acceptability (7.35 to 6.54) respectively. In the same way the mean sensory scores decreased significantly from initial to 6th day in whole rice bran and stabilized whole rice bran incorporated cakes.

In 10% whole rice bran incorporated cakes the sensory score decreased from 6.29 to 5.39 for color, 6.79 to 5.23 for texture, 6.00 to 5.38 for taste, 5.79 to 5.28 for flavor and 6.42 to 5.62 for overall acceptability. In 10% stabilized whole rice bran incorporated cakes the sensory score decreased from 6.93 to 6.38 for color, 7.21 to 6.00 for texture, 6.50 to 6.07 for taste, 6.35 to 5.85 for flavor and 6.42 to 6.23 for overall acceptability.

Statistical analysis revealed a significant difference for all the sensory characteristics of cakes between the treatments and both before and after storage period of 0th, 3rd and 6th days.

Table 4.17: Mean sensory scores of cakes on storage

Treatment	Duration (Days)	Sensory attributes				
		Color	Texture	Taste	Flavor	Overall acceptability
T0	0 days	7.36 ^a ±1.22	7.21 ^a ±0.89	7.07 ^a ±1.00	7.14 ^a ±0.86	7.35 ^a ±0.63
	3 days	7.25 ^a ±0.97	6.42 ^a ±0.51	6.67 ^a ±0.78	6.50 ^a ±1.09	6.83 ^a ±0.72
	6 days	6.92 ^a ±0.86	6.31 ^a ±0.85	6.54 ^a ±0.78	6.08 ^a ±0.64	6.54 ^a ±0.52
T1	0 days	6.29 ^{bc} ±0.99	6.79 ^{ab} ±0.70	6.00 ^c ±0.85	5.79 ^b ±0.70	6.42 ^{bc} ±0.66
	3 days	5.58 ^c ±0.90	5.33 ^c ±0.78	5.41 ^c ±0.90	5.50 ^{bc} ±0.90	6.08 ^{bc} ±0.67
	6 days	5.39 ^c ±0.85	5.23 ^c ±0.83	5.38 ^c ±0.65	5.28 ^{bc} ±0.87	5.62 ^c ±0.77
T2	0 days	6.93 ^{ab} ±0.92	7.21 ^{ab} ±0.80	6.5 ^{ab} ±0.76	6.35 ^b ±1.08	6.42 ^b ±0.76
	3 days	6.50 ^{ab} ±1.17	6.16 ^{ab} ±0.83	6.25 ^{ab} ±0.87	6.00 ^{ab} ±1.04	6.00 ^{ab} ±0.67
	6 days	6.38 ^{ab} ±0.65	6.00 ^{ab} ±0.71	6.07 ^{ab} ±0.76	5.85 ^{ab} ±0.99	6.23 ^{ab} ±0.83
CD Value	0 days	0.715	0.672	0.666	0.682	0.531
	3 days	0.795	0.643	0.709	0.800	0.578
	6 days	0.631	0.617	0.552	0.650	0.583

Note:

- C- Control sample.
- T1- Cakes prepared by incorporated 10% of whole rice bran.
- T2- Cakes prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.6.6 Nutritional characteristics of cakes during storage

The moisture content of cakes before storage was 14.80%, 14.05% and 13.86% for the control, T1 and T2 respectively. The moisture content of cakes after storage was 14.84%, 14.12% and 13.94% for the control, T1 and T2 respectively.

The results revealed that there was significant increase in the mean moisture content of cakes from 0th day to 6th day of storage. The increase in moisture content of cakes might be due to hygroscopic nature of rice bran and wheat flour.

The protein content of cakes before storage was 7.88%, 8.46% and 8.68% for the control, T1 and T2 respectively. The protein content of cakes after storage was 7.68%, 8.36% and 8.64% for the control, T1 and T2 respectively.

The results revealed that there was a significant decrease in the mean protein content of cakes from 0th day to 6th day of storage period as shown in the Table 4.18. The decrease in protein content during storage might be due to hydrolysis of peptide bonds by the help of protease enzyme that cause splitting of protein molecules during storage.

The ash content of cakes before storage was 0.75%, 1.59% and 1.34% for the control, T1 and T2 respectively. The ash content of cakes after storage was 0.65%, 1.30% and 1.32% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in the mean ash content of cakes from 0th day to 6th day of storage as shown in the Table 4.18.

The crude fibre content of cakes before storage was 0.88%, 1.24% and 1.13% for the control, T1 and T2 respectively. The crude fibre content of cakes after storage was 0.75%, 1.20% and 1.07% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in the mean crude fibre content of cakes from 0th day to 6th day of storage as showed in the Table 4.18. The decrease in crude fiber might be due to the degradation of hemicelluloses and other structural polysaccharides during storage. Similar decline in crude fiber content was reported by Singhet *al.*, (2006) in blanched and malted pearl millet incorporated cakes.

The fat content of cakes before storage was 19.35%, 22.83% and 22.69% for the control, T1 and T2 respectively. The fat content of cakes after storage was 19.22%, 21.89% and 22.58% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in mean fat content of cakes from 0th day to 6th days of storage as shown in the Table 4.18. This decrease in fat content during storage was due to the incorporation of moisture in cakes from the atmosphere and due to oxidation of fatty acids resulting in free fatty acid formation.

The carbohydrate content of cakes before storage was 50.74%, 50.27% and 49.53% for the control, T1 and T2 respectively. The carbohydrate content of cakes after storage was 50.35%, 50.01% and 49.37% for the control, T1 and T2 respectively. The results revealed that there was a significant decrease in the mean carbohydrate content of cakes from 0th day to 6th day of storage as shown in the Table 4.18.

Table 4.18: Nutritional characteristics of cakes during storage

Nutrients	Duration	C	T1	T2	CD value
Moisture (%)	Before	14.80 ^a ±0.37	14.05 ^b ±0.08	13.86 ^b ±0.14	0.389
	After	14.84 ^a ±0.85	14.12 ^b ±0.58	13.94 ^b ±1.05	0.387
Protein (%)	Before	7.88 ^a ±0.19	8.46 ^b ±0.22	8.68 ^b ±0.08	0.291
	After	7.68 ^a ±0.36	8.36 ^b ±0.21	8.64 ^{bc} ±0.11	0.416
Ash (%)	Before	0.75 ^a ±0.07	1.59 ^b ±0.13	1.34 ^b ±0.12	0.249
	After	0.65 ^a ±0.07	1.30 ^b ±0.15	1.32 ^c ±0.06	0.195
Crude fiber (%)	Before	0.88 ^a ±0.07	1.24 ^b ±0.10	1.13 ^b ±0.04	0.122
	After	0.75 ^a ±0.08	1.20 ^b ±0.08	1.07 ^c ±0.10	0.140
Fat (%)	Before	19.35 ^a ±0.07	22.83 ^b ±0.09	22.69 ^b ±0.16	0.208
	After	19.22 ^a ±0.18	21.89 ^b ±0.40	22.58 ^c ±0.07	0.430
Carbohydrate (%)	Before	50.74 ^a ±0.07	50.27 ^b ±0.05	49.53 ^b ±0.89	0.914
	After	50.35 ^a ±0.10	50.11 ^a ±0.15	49.37 ^b ±0.48	0.536

Note:

- C- Control sample.
- T1- Cakes prepared by incorporated 10% of whole rice bran.
- T2- Cakes prepared by incorporated 10% of stabilized whole rice bran.
- CD- Critical difference.
- Values are expressed as mean ± standard deviation of three determinations.
- Mean values with similar superscripts within a row do not differ significantly (P<0.05).

4.7 Microbial quality of baked products during storage

The best accepted products from sensory evaluation *i.e.* biscuit (WRB 10% and SWRB 10%), cookies (WRB 10% and SWRB 20%) and cakes (WRB 10% and SWRB 20%) were stored in polythene covers and microbial load was assessed.

In biscuits and cookies the microbial count such as total bacterial count and total fungal count was observed on 0th, 15th and 30th days. In cakes the total bacterial count and total fungal count was observed on 0th, 3rd and 6th days (Table 4.19).

The total bacterial count in control biscuits was 1×10^3 CFU on 30th day, T1 had 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days and T2 had 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days.

The total bacterial count in control cookies was 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days, in T1 had 2×10^3 CFU and 3×10^3 CFU on 15th and 30th days and T2 had 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days.

The total bacterial count in control cake was 1×10^3 CFU and 2×10^3 CFU on 3rd and 6th days, in T1 had 2×10^3 CFU & 3×10^3 CFU on 3rd & 6th days and T2 had 1×10^3 CFU and 2×10^3 CFU on 3rd and 6th days.

The total fungal count in control biscuits was 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days, in T1 had 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days and T2 had 1×10^3 CFU on 30th days.

The total fungal count in control cookies was 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days, in T1 had 1×10^3 CFU and 2×10^3 CFU on 15th and 30th days and T2 had 1×10^3 CFU on 30th days.

The total fungal count in control cakes was 1×10^3 CFU and 2×10^3 CFU on 3rd and 6th days, in T1 had 2×10^3 CFU and 3×10^3 CFU on 3rd & 6th days and T2 had 2×10^3 CFU & 2×10^3 CFU on 3rd & 6th days.

The total bacteria and fungi count was observed in the products during storage *i.e.* in biscuit and cookies at 15th and 30th days. In case of cake presence of total bacteria and fungi from 3rd to 6th day of storage period was observed. This may be due to moisture content of products and also storage condition of the products.

The total bacteria and fungi count was more in whole rice bran incorporated baked products compared to control and stabilized whole rice bran incorporated baked products.

The increase in microbial load as the storage period lengthened might have been due to a corresponding increase in moisture content during storage period but the total load for bacterial count and fungi were within the permissible limits. This result agreed with the reported by Nagiet *al.*, (2012) in cereal bran incorporated biscuits.

Table 4.19: Microbial load in baked products during storage

Products	Storage period (Days)	Total bacterial count ($\times 10^3$ CFU)			Total fungal count ($\times 10^3$ CFU)		
		C	T1	T2	C	T1	T2
Biscuit	0	Nil	Nil	Nil	Nil	Nil	Nil
	15	Nil	1.00	1.00	1.00	1.00	Nil
	30	1.00	2.00	2.00	2.00	2.00	1.00
Cookies	0	Nil	Nil	Nil	Nil	Nil	Nil
	15	1.00	2.00	1.00	1.00	1.00	Nil
	30	2.00	3.00	2.00	2.00	2.00	1.00
Cakes	0	Nil	Nil	Nil	Nil	Nil	Nil
	3	1.00	2.00	1.00	1.00	2.00	2.00
	6	2.00	3.00	2.00	2.00	3.00	2.00

Note:

- C- Control sample.
- T1- Baked products *i.e.* biscuits, cookies and cakes prepared by incorporated 10% of whole rice bran.
- T2- Baked products *i.e.* biscuits and cakes prepared by incorporated 10% of stabilized whole rice bran and cookies prepared by incorporated 20% of stabilized whole rice bran

Chapter V

SUMMARY AND CONCLUSIONS

Rice is a staple food for approximately 70% of the world's population. Over 600 million tons of rice is harvested annually on a global basis. Much of the nutritional value lies in the bran and germ which has traditionally been discarded during milling processes. Rice bran is obtained as a by-product during the rice milling process and the outer layer is removed at the time of polishing of husked rice.

Rice bran has a range of bioactive phyto-chemicals with potential for reducing the risk of chronic degenerative diseases. Rice bran is a good source of protein, minerals, fatty acids and dietary fibre content. Addition of dietary fibre to a wide range of products will contribute to the development of value-added foods or functional foods that currently are in high demand. Processing of rice bran has improved the nutritional, functional and keeping quality of rice bran. Healthy complex carbohydrates found in stabilized rice bran have "hypoglycemic effect".

Therefore, a study was planned to develop and evaluate bakery products by using wheat flour and rice bran at three different levels (100:0, 90:10, 80:20 & 70:30) with two types of rice bran (WRB and SWRB).

The salient finding of the study are given here under:

- The nutritional quality characteristics of whole rice bran (WRB) and stabilized whole rice bran (SWRB) indicated that the moisture, protein, ash, fiber, fat, carbohydrate, calcium and iron were 10.77% and 7.11%, 15.66% and 16.28%, 9.63% and 8.29%, 9.03% and 8.07%, 20.47% and 19.11%, 38.62% and 34.41%, 50.57mg and 54.15mg, 5.46mg and 7.05mg respectively.
- The functional quality characteristics of whole rice bran (WRB) and stabilized whole rice bran (SWRB) indicated that the bulk density was in the range of 0.31-0.24 g/ml and water absorption capacity was in the range of 2.17-2.33 ml/g.
- The color values for WRB and SWRB i.e. L* value was significantly higher ($P > 0.05$) for WRB (62.77 ± 0.44) compared to the SWRB (58.73 ± 0.78). The a* and b* values was

significantly lower ($P>0.05$) for the WRB (1.81 ± 0.24 and 18.22 ± 0.65) compared to the SWRB (2.48 ± 0.15 and 20.33 ± 0.87).

- Three baked products (biscuit, cookies and cakes) were developed by incorporating three different levels (10%, 20% and 30%) of WRB and SWRB.
- Mean sensory scores revealed that biscuit and cakes were best accepted at 10 percent level of WRB and SWRB incorporation. Cookies were best accepted at 10 per cent in WRB and 20 per cent in SWRB respectively.
- The incorporation of rice bran at three different levels had a significant effect on physical quality characteristics of the biscuits, cookies and cakes.
- The diameter of the biscuits and cookies was 41.07 and 58.65mm, 40.83 and 55.81mm, 40.00 and 54.04mm for the control, T1 and T2 respectively.
- The thickness of the biscuits and cookies was 7.88 and 15.60mm, 8.61 and 15.86mm, 8.87 and 16.06mm for the control, T1 and T2 respectively.
- The spread ratio of the biscuits and cookies was 5.21 and 3.51, 4.74 and 3.51, 4.51 and 3.36 for the control, T1 and T2 respectively.
- The height of the cake was 2.23cm, 1.93cm and 2.06cm for the control and 10% of whole rice bran and stabilized whole rice bran incorporated cakes respectively.
- The length of the cake was 15.00cm, 14.16cm and 14.66cm for the control and 10% of whole rice bran and stabilized whole rice bran incorporated cakes respectively.
- The width of the cake was 4.50cm, 4.30cm and 4.50cm for the control and 10% of whole rice bran and stabilized whole rice bran incorporated cakes respectively.
- The volume of the cake was 150.52cm, 117.51cm and 135.89cm for the control and 10% of whole rice bran and stabilized whole rice bran incorporated cakes respectively.
- The density of the cake was 1.14 kg/cm^3 , 1.08 kg/cm^3 and 1.06 kg/cm^3 for the control and 10% of whole rice bran and stabilized whole rice bran incorporated cakes respectively.
- Statistically significant difference was observed in all the chemical and nutritional quality characteristics (moisture, protein, fat, carbohydrate, crude fibre and ash) of baked products.
- The increase in nutritional quality parameters (protein, fat, crude fibre, carbohydrate and ash) with increase in the level of incorporation of WRB and SWRB was observed.

- There was a decreasing trend of sensory scores for color, texture, taste, flavor, and overall acceptability for control, raw rice bran and stabilized rice bran incorporated bakery products, during shelf life study.
- The bacteria and fungi count was observed in the products at the end of the storage period *i.e.* in biscuit and cookies on 30th day. In case of cake there was a presence of total bacteria and fungi from 3rd to 6th day of storage period, thus the products were microbiologically safe throughout the storage period.

It was concluded that the stabilized rice bran can be successfully incorporated in bakery products as it enhances the nutritional quality, functional properties and shelf life of the baked products. Utilization of dietary fibre from rice bran needs more exploitation as it is a by-product from food processing industry, discarded as waste or used as animal feed. The result on stabilization of rice bran was suitable for the development of products since it was acceptable organoleptically and also rich in macro and micro nutrients and had good functional properties. The dietary fibre has been claimed as a functional ingredient which is useful in nutraceutical formulations in the management of life style disorders.

Recommendations for future research

- ❖ Rice bran can be incorporated into other Indian food products to increase the nutritive value.
- ❖ Nutraceutical food products can be developed by the process of incorporation of stabilized rice bran to prevent life style diseases such as obesity, diabetes, high blood pressure and colon cancer etc.

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APPENDIX-I

Preparation of biscuit (per 100g)

Ingredients	Quantity (g)
Refined wheat flour	50
Sugar powder	25
Fat	30
Baking powder	Pinch
Vanilla essence	Few drops

Procedure:

- Sieve flour and baking powder together.
- Cream fat and sugar till light and fluffy.
- Add sieved flour to the cream and make stiff dough.
- Divide the dough into small portions.
- Round them and place on baking trays.
- Bake them at 200⁰ C till golden brown and crisp.

APPENDIX-II

Preparation of cookies (per 100g)

Ingredients	Quantity (g)
Refined wheat flour	50
Sugar powder	25
Fat	30
Salt (tsp)	1/4
Baking powder	Pinch
Egg	1
Milk	Little to mix

Procedure:

- Sieve flour, salt and baking powder together.
- Cream fat and sugar, beat egg to mix.
- Add sieved flour and milk to the cream and make stiff dough.
- Divide the dough into uniform sized balls.
- Round them and cut with cookies cutters.
- Placed on a greased baking tray.
- Bake them at 190⁰ C for 15 minutes.
- Cooling, packaging and storage.

APPENDIX-III

Preparation of cakes (per 100g)

Ingredients	Quantity (g)
Refined wheat flour	40
Sugar powder	25
Fat	35
Egg	1
Baking powder	Pinch
Clove (nos)	2
Cardamom (pieces)	3
Cinnamon (pieces)	3
Vanilla essence	Few drops

Procedure:

- Prepare the caramelized syrup by heating the sugar with water.
- Sieve flour, sugar and baking powder together.
- Cream fat and sugar and beat egg to mix.
- Add sieved flour, essence and caramel syrup.
- Add all ingredients and mix thoroughly.
- Pour the batter into pan that you previously greased.
- Place the cake in pre heated oven.
- Bake them at 180⁰ C for 1 hour.
- Cooling, packaging and storage.

APPENDIX-IV

SCORE CARD FOR SENSORY EVALUATION OF BAKED PRODUCTS

Directions: Please taste the samples one by one and evaluate them for the following parameters on hedonic scale as given at the end of form. It is very important to rinse mouth thoroughly with clean water after evaluation of each sample.

Name of the judge: _____

Age: _____

Sex: Male /Female

Signature: _____

Date: _____

Attributes	Samples			
Color				
Texture				
Taste				
Flavor				
Overall Acceptability				

Hedonic scale

- 9 Like extremely
- 8 Like very much
- 7 Like moderately
- 6 Like slightly
- 5 Neither like nor dislike
- 4 Dislike slightly
- 3 Dislike moderately
- 2 Dislike very much
- 1 Dislike extremely

APPENDIX-V

Estimation of moisture

The moisture content of the samples was determined by following the method of AOAC (1990)

Procedure

A petridish was weighed with lid (W_1) and approximately 10g of sample was weighed into it and weighed again (W_2). Petridish along with sample was dried in an oven at 100-105⁰C for 15-17hrs and cooled in a desiccator and weight was noted (W_3). The same process was repeated till constant weight was obtained.

Calculations

$$\text{Moisture (\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{(W_2 - W_1)} * 100$$

APPENDIX-VI

Estimation of protein

The protein content of the samples was determined by following the method of AOAC (2005)

Procedure

1. Set the pressure of helium gas and dry air cylinders at 2.7 bar
2. Set the optimum conditions for the air compression i.e. 2.7 bar
3. Switch on the software and main instrument, put on the gases on and place in stand by mode
4. Let the Lecoanalyser warm up for one to two hours
5. Go to diagnostics option, select ambient monitor, see for the system parameters as follows and should be in the range limit as detailed
6. When the instrument is ready, create a method for purging, burning and filling the ballet for combustion of the sample
7. Enter the blank weight by default as 0.500 and press analyse button. Automatically protein percent will be displayed
8. Repeat blanks until the instrument is stabilized. Blank correction can be done
9. Analyse standard EDTA (ethylene tetra diamine) four to five times, standard corrected values range is 9.40 to 9.70% nitrogen. Standards should be calibrated when standard corrected value falls within range.
10. Analyse samples in triplicate and note the average protein content in g percent.

APPENDIX-VII

Estimation of fat

The fat content of the samples was determined by using the method of AOAC (1981)

Principle

The extraction of groups of chemical constituents having similar chemical and physical properties is widely used in food analysis. Fats are characterized by the readiness with which they are extracted by ethyl ether, petroleum spirit, carbon disulphide etc. Hence these organic solvents are used for extracting fats from food samples.

Procedure

1. Make a thimble with whatmann filter paper No:1 and take the weight (W1)
2. Add about 5 g of the sample to the thimble, close it and take the weight (W2)
3. Place the thimble in a soxhlet extractor and extract with petroleum ether (B.P.60-80⁰ C) for about 16 hrs
4. Remove the thimble, dry for 1hr to eliminate the organic solvent present in the thimble and take the weight of the thimble (W3)
5. Filter and transfer the petroleum ether extract (2to3 times) into weighed 250ml beaker (A). wash the soxhelet flask containing the ether extract 2 to 3 times with 5ml of petroleum ether every time and transfer the wastings also to the 250ml beaker
6. Evaporate petroleum ether in the 250ml beaker completely and dry the residue in an oven at 60-80⁰C. cool in a dessicator and take the weight of the beaker(B)

Calculation

$$\text{Fat\%} = \frac{\text{Fat content of the sample}}{\text{Intial weight of the sample}} * 100$$

APPENDIX-VIII

Estimation of crude fibre

The fibre content of the samples was determined by using the method of AOAC (1990)

Principle

The samples were allowed to boil with dilute H_2SO_4 (1.25%) and dilute NaOH (1.25%) and the remaining residue after these digestions is taken as crude fibre.

Procedure

1. Put 1gm of moisture and fat free sample in the fibre bags
2. Put the glass spacer into the bags
3. Load the bag in the sample carousel axial on the previewed position of the hot plate
4. Creating a method to estimate crude fibre
 - Double click on the menu
 - Press enter for method definition
 - Enter method number from 0-9
 - Select detergent H_2SO_4
 - Add volume 1.31 by pressing plus or minus enter
 - Heating power 45%
 - Circulation time 10 sec enter
 - Circulation time pause 30 sec enter
 - Boiling time 30 min enter
 - Suction 2 min enter
 - Rising 2 min enter
 - Select detergent NaOH
 - Add volume 1.31 enter
 - Circulation time 10 sec
 - Circulation pause 20 sec
 - Boiling time 30 min enter
 - Suction 2 min enter
 - Rising 2min enter

- Select detergent H_2O
 - Add volume 1.31 enter
 - Circulation time 10 sec enter
 - Circulation pause 15 sec enter
 - Boiling time 5 min enter
 - Suction 2 min enter
5. After defining all programme parameter for the individual boiling phases, the total duration of the method is displayed
 6. Press key “ESC” to return to “fibretherm menu” the programme is finished and stored
 7. Start the programme in the fibretherm
 8. After completion of the programme, remove the fibre bags
 9. Transfer the residue to weighed crucible (W1) and dry over night at $80^{\circ}C$ - $100^{\circ}C$ and take the weight (W2)
 10. Heat the crucible in a muffle furnace at $600^{\circ}C$ for 2-3 hrs
 11. Cool in the dessicator and take weight of the crucible again (W3)

Observations

Weight of the sample = W1 gm

Weight of the crucible + sample before heating at $600^{\circ}C$ = W2 gm

Weight of the crucible + sample after heating at $600^{\circ}C$ = W3 gm

Weight of the crude fibre = (W2-W3) gm

Calculations

$$\text{Crude fibre (g \%)} = \frac{100 - (\text{moisture} + \text{fat}) * \text{wt of fibre}}{\text{wt of sample taken (moisture \& fat free, W1)}}$$

APPENDIX-IX

Estimation of ash

The ash content of the samples was determined by using the method of AOAC (1984)

Procedure

Weigh accurately about 5gms of sample in a tared silica/platinum dish. Char the material carefully on a burner and transfer the dish to a muffle furnace and ash at a temperature of $550 \pm 10^{\circ}$ C until the ash is free of carbon. Heat the dish again at $550 \pm 10^{\circ}$ C for 30 minutes. Cool in a dessicator and weigh. Repeat this process of heating for 30 minutes, cooling in a dessicator and weighing until the difference between two successive weighing is less than 1mg. record the lowest weight.

Calculation

$$\text{Total ash (\% on dry weight)} = \frac{(W_2 - W) * 100 * 100}{(W_1 - W) * (100 - M)}$$

Where

W₁= Weight in gms of silica dish+sample

W₂= Weight in gms of silica dish+ash

W = Weight in gms of empty silica dish

M = Moisture % of the sample

APPENDIX – X

Estimation of carbohydrates

Estimation of carbohydrates in the samples was carried out by Anthrone method (AOAC, 1981).

Reagents:

1. 2.5 N HCl
2. Anthrone reagent: Dissolve 200mg of Anthrone in 100ml ice cold 95% H₂SO₄ .
3. Stock standard glucose solution: Dissolve 100mg of glucose in 100ml of distilled water (1mg/ml).
4. Working standard solution: Dilute 10ml of stock standard solution to 100ml with distilled water.

Procedure: 100mg of sample was weighed and placed in boiling test tube. Then the sample was hydrolyzed by keeping it in a boiling water bath for 3 hrs with 5ml 2.5N HCl and cool to room temperature. It was neutralized with solid Na₂CO₃ until the effervescence ceased. The volume was made up to 100ml and centrifuged to collect the supernatant and took 0.5ml and 1ml aliquots. The standards were prepared with concentration 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml along with a blank and the volume was made up to 1ml in all test tubes, then 4ml of Anthrone reagent was added followed by 8min heating in boiling water bath. After cooling, the red green color was read at wavelength of 630nm. The standard curve was plotted with concentration on X-axis and absorbance on Y-axis. From the standard graph, amount of carbohydrate present in sample was calculated.

Calculation:

$$\text{Amount of carbohydrates present} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

APPENDIX-XI

Estimation of calcium

The calcium content of the samples was determined by using the method of AOAC (2000)

Reagents:

- 1) 0.01N KMnO_4 : 31.6mg of KMnO_4 was weighed into a 100ml volumetric flask and added distilled water and made upto 100ml.
- 2) 0.01 N Oxalic acid: 63 mg of oxalic acid was weighed into a 100ml volumetric flask, distilled water was added and makeup the volume 100ml.
- 3) 2 N H_2SO_4 : Take 5.6ml of concentrated H_2SO_4 and add distilled water slowly to the acid and make up the volume to 100ml.
- 4) Standardize 0.01 KMnO_4 (5ml): Against 0.01N oxalic acid using 2ml of 2N H_2SO_4 (Calculate the normality and make necessary correction).
- 5) 0.1% Methyl red indicator: 10mg of methyl red indicator was dissolved in 10ml of ethanol.
- 6) 20% ammonia: 20ml of ammonia was dissolved in distilled water and made upto 100ml
- 7) 10% acetic acid: 10ml of acetic acid was dissolved in distilled water and made upto 100ml.
- 8) 6% ammonium oxalate: 6g ammonium oxalate, was added in distilled water, dissolve and made upto 100ml (slightly warm the solution if necessary).
- 9) Wang's wash: Mix 32.7ml ethanol and 2.3ml ammonia solution.

Procedure:

- 1) 5 ml of mineral solution was taken in a 15 ml centrifuge tube. Add 2 ml water and a drop of methyl red indicator.
- 2) Ammonium hydroxide was added drop wise until the pink colour disappears and then adds acetic acid drop wise until faint pink colour appears.
- 3) Shake the solution well and add 1 ml of 6% ammonium oxalate. Mix thoroughly and it was allowed to stand for 1hour.
- 4) Centrifuge the tube and the tube was invert on a blotting paper for 5 min.

- 5) The precipitate was washed with 4 ml of wang's wash solution thoroughly and centrifuging process was repeated.
- 6) Precipitate was dissolved in 2 ml of 2 N H₂SO₄. The solution was heated in a water bath up to 70-75°C and titrate against 0.01N KMnO₄ (while the solution is still hot) until a faint pink colour appears.

Readings to be recorded:

- Total volume of mineral solution
- Volume used for estimation
- Weight of the sample taken for ashing
- Titre value

Calculation: Titre value of 1.0 ml of KMnO₄

$$\text{Titre value} \times \frac{\text{Total volume of mineral solution}}{\text{Volume used for estimation}} \times \frac{100}{\text{wt. of the sample taken for ashing}} = \text{mg/100}$$

APPENDIX-XII

Estimation of iron

The iron content of the samples was determined by using the method of AOAC (2000)

Principle

Organic matter in the sample is destroyed by ashing and the resulting ash is dissolved in hydrochloric acid and diluted to a known volume with water. Whole of the iron present in the aliquot of ash solution is reduced with hydroxylamine hydrochloride and the e (II) is determined spectrophotometrically as its colored complex with, α - α -dipyridyl, the solution being buffered with acetate buffer solution. Absorption of the resulting complex is read at 510 nm.

Procedure

Pipette 10 ml aliquot of ash solution into 25 ml volumetric flask, and add 1 ml hydroxylamine hydrochloride solution. After 5 min, add 5 ml buffer solution and 1 ml O - phenanthroline solution or 2 ml of dipyridyl solution and dilute to volume. Determine absorbance of solution at 510 nm. From absorbance reading, determine Fe content present in aliquot of ash solution taken by referring to standard curve.

Preparation of Standard Curve:

Pipette 0.0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml of Fe standard solution {F-ii} into a series of 25 ml volumetric flasks and add to each of them exactly 0.2 ml of conc. HCl. Dilute each of them to exactly 10 ml with water, and then add reagents in the same way as for the sample, Plot the quantity of Fe (in mg) against the absorbance.

Calculations:

Iron content of sample (Mg Fe / 100gm sample) =

$$\frac{\text{Quantity of Fe in aliquot of ash solution (From calibration curve)}}{\text{Aliquot of ash solution taken for determination}} \times \frac{\text{total volume of ash solution}}{\text{Wt. of the sample}} \times 100$$

