DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL

BARBHAI MRUNAL D.

M.Sc. (Agri.) Food Science and Nutrition

DOCTOR OF PHILOSOPHY IN HOME SCIENCE

(FOODS AND NUTRITION)

2020

DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL

BY

BARBHAI MRUNAL D.

M.Sc. (Agri.) Food Science and Nutrition

THESIS SUBMITTED TO THE PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY IN HOME SCIENCE

(FOODS AND NUTRITION)

CHAIRPERSON: Dr. T.V. HYMAVATHI

DEPARTMENT OF FOODS AND NUTRITION

POST GRADUATE AND RESEARCH CENTRE

RAJENDRANAGAR, HYDERABAD – 500 030

PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY 2020

DECLARATION

I, **BARBHAI MRUNAL D.** hereby declare that the thesis entitled **"DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL"** submitted to **Professor Jayashankar Telangana State Agricultural University** for the degree of **Doctor of Philosophy in Home Science** 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: Hyderabad **(BARBHAI MRUNAL D.)**

Date: **I.D. No. HHD/2017-05**

CERTIFICATE

Ms. BARBHAI MRUNAL D. has satisfactorily prosecuted the course of research and that thesis entitled **"DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL"** 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.

(Dr. T. V. HYMAVATHI)

Date: **(Chairperson)**

CERTIFICATE

This is to certify that the thesis entitled **"DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL"** submitted in partial fulfillment of the requirements for the degree of '**DOCTOR OF PHILOSOPHY IN HOME SCIENCE'** of the Professor Jayasahankar Telangana State Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Ms. BARBHAI MRUNAL D.** 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 and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

Chairman	Dr. T. V. Hymavathi Professor and University Head (Foods and Nutrition) Department of Foods & Nutrition, Post Graduate & Research Centre, PJTSAU, Rajendranagar, Hyderabad – 500030	________________
Member	Dr. K. Aparna Senior Scientist and Head (Foods and Nutrition) MFPI, Quality Control Laboratory, EEI campus, PJTSAU, Rajendranagar, Hyderabad – 500030	________________
Member	Dr. M. Sreedhar Principal Scientist and Head Regional Rice and Sugarcane Research Centre, PJTSAU, Rudrur – 503188	________________
Member	Dr. V. Sudha Rani Director, Extension Education Institute, PJTSAU, Rajendranagar, Hyderabad – 500030	________________
External – Examiner of final viva voce	Dr. Usha Ravindra Professor, Department of Food Science and Nutrition, University of Agricultural Sciences, GKVK, Bangalore – 560065	________________

Date of final viva-voce: 10-03-2021

ACKNOWLEDGEMENT

I would first like to thank God for all the strength that helped me in successfully completing my research work.

I sincerely want to express my gratitude and respect to chairman of my Advisory committee **Dr. T. V. Hymavathi**, Professor and University Head, Department of Foods and Nutrition, PJTSAU, Hyderabad for her guidance, encouragement, constant supervision, constructive criticism and support throughout the period of my research. It was a great to be associated with her and having her as my major advisor.

I am extremely grateful to all the members of my advisory committee **Dr. Aparna Kuna,** Senior Scientist and Head (Foods and Nutrition) MFPI – Quality Control Lab, PJTSAU, Hyderabad, **Dr. M. Sreedhar**, Principal Scientist and Head, Regional Rice and Sugarcane Research Centre, PJTSAU, Rudrur and **Dr. V. Sudha Rani,** Director, Extension Education Institute, PJTSAU, Hyderabad for providing guidance, suggestions and directions throughout the thesis work.

Many thanks to **Mrs. Pradeepa Robert, Ms. V. Thejashri** and the entire staff of **Millet Processing and Incubation Centre** (MP&IC) for providing necessary facilities and helping hand during the study.

I would also take this opportunity to thank the entire staff of Central Instrumentation Cell (CIC), **Mr. M.J.R. Raju, Mrs. G. Saranya, Mrs. D. Sridevi and Mr. T. Joseph** for their kind help and assistance all the time. With the availability of the equipment's in CIC conducting experiments for this research work was easier and accurate.

My special thanks to **Dr. Venkateshwaralu** from Indian Institute of Millet Research, for allowing the use of his laboratory and **Dr. Neeharika** for her help while conducting the mineral estimation. Also, I thank **Dr. Rama Rao**, and the staff from Nutrition Laboratory, ICAR-Directorate of Poultry Research, for providing laboratory facilities to conduct experiment related to blood stress profile.

My heartfelt thank staff of Health Centre, PJTSAU for providing assistance during the supplementation study. Special thanks to lab technician **Mr. Sudheer**, for collection of blood samples during the supplementation study.

I am grateful and acknowledge teachings, support and cooperation of all my teachers ***Dr. K. Uma Maheswari****,* ***Dr. V. Vijayalakshmi, Dr. T. Kamalaja, Dr. Sucharitha Devi, Dr. B. Anila Kumari, Dr. W. Jessie Suneetha****,* ***Ms. E. Jyothsna*** *and* ***Dr. G. Nageswara Rao*** *during the course work. A warm thanks to all the non-teaching and office staff for their help during the study period.*

This study was impossible without the cooperation and sincerity of all the subjects from BSc (Ag.) who participated in the study and helped me conduct the work successfully.

I extend my sincere gratitude to **University Grant Commission** *for providing UGC-JRF fellowship throughout my degree programme.*

Family is the backbone of everyone and take the honour in thanking mine. All this was only possible because of round-the-clock moral support, love and strength given by my mother **Madhuri Khapre***, aunty* ***Mrs. Prajakta Khapre****, uncle* ***Mr. Padmanabh Khapre*** *and brother* **Omkar Khapre.** *I am also fortunate to have true friends* ***Vidya Ghule, Shruthy K. S., Vijaykumar Waghmare, Vikram K. V.*** *and* ***Shriniketan Puranik*** *who unconditionally supported stood with me in all the situations.*

*I also thank my classmate (***Mounika***), seniors (****Bhuvana N. and Manju Yadav****), juniors (****Merina, Precious, Roshita, Jelang, Srilekha, Jyooti, Reema, Sravanthi, Santhisirisha, and Sreeja****) for making stay at Hyderabad fun and comfortable.*

Finally, thanks to all who knowingly, unknowingly helped me, made this journey possible and pleasant. Kindly consider that any omissions in acknowledgement does not mean lack of appreciation.

Place: Hyderabad

Date: **(BARBHAI MRUNAL D.)**

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

A. CHO	:	Available carbohydrate
ANOVA	:	Analysis of Variance
BLR	:	Baking loss rate
BM	:	Barnyard millet bran
BSM	:	Kodo millet bran stabilized with MW2
CF	:	Crude fiber
Cfu	:	Colony forming units
CHO	:	Carbohydrate
CRD	:	Complete randomized design
DD	:	Degree of dehulling
Df	:	Degree of freedom
DPPH	:	2, 2-Diphenyl-1-picrylhydrazyl
FC	:	Foaming Capacity
FFA	:	Free fatty acid
FM	:	Foxtail millet bran
FRAP	:	Ferric Reducing Antioxidant Power
FSM	:	Foxtail millet bran stabilized with MW2
GAE	:	Gallic Acid Equivalent
GI	:	Glycemic index
GL	:	Glycemic load
GSH	:	Glutathione
HCA-AXs	:	Hydroxycinnamic acid bound arabinoxylans
HDL	:	High Density Lipoprotein
HT1	:	Hot air oven treatment (100ºC for 3 hours)
HT2	:	Hot air oven treatment (130ºC for 20 minutes)
iAUC	:	incremental area under the curve
KM	:	Kodo millet bran
KSM	:	Barnyard millet bran stabilized with MW2
LDL	:	Low Density Lipoprotein
MDA	:	Malondialdehyde
MT	:	Million Tonnes

MW1	:	Microwave heating (1.5 minutes at 900 Watt)
MW2	:	Microwave heating (2.5 minutes at 900 Watt)
OAC	:	Oil Absorption Capacity
ORAC	:	Oxygen Radical Absorbance Capacity
PA	:	Phytic Acid
PBR	:	Percent bran recovery
PM	:	Proso millet bran
PSM	:	Proso millet bran stabilized with MW2
PV	:	Peroxide values
RBC	:	Red Blood Cell
RDA	:	Recommended dietary allowances
RE	:	Retinol equivalent
ROS	:	Reactive oxygen species
SD	:	Standard Deviation
SE	:	Standard error
SEm	:	Standard error of mean
T.CHO	:	Total Carbohydrate
TBA	:	Thiobarbituric acid
TBARS	:	Thiobarbituric acid reactive substances
TBC	:	Total bacterial count
TC	:	Total cholesterol
TDF	:	Total Dietary Fiber
TE	:	Trolox equivalent
TFC	:	Total Flavonoid Content
TG	:	Triglycerides
TMC	:	Total mould count
TP	:	Total phenols
TPC	:	Total phenolic content
TPTZ	:	2,4,6-tripyridyl-s-triazine
VLDL	:	Very Low Density Lipoprotein
WAC	:	Water Absorption Capacity
WSI	:	Water Solubility Index

Author	**:**	**BARBHAI MRUNAL D.**
ID No.	**:**	**HHD/2017 – 05**
Title of the Work	**:**	**DEVELOPMENT OF DESIGNER PRODUCTS BY INCORPORATING MILLET BRAN AND ASSESSING THEIR THERAPEUTIC POTENTIAL**
Degree	**:**	**DOCTOR OF PHILOSOPHY IN HOME SCIENCE**
Faculty	**:**	**HOME SCIENCE**
Department	**:**	**FOODS AND NUTRITION**
Chairperson	**:**	**Dr. T. V. HYMAVATHI**
University	**:**	**PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY**
Year of submission	**:**	**2020**

ABSTRACT

World over there is a tremendous demand for bakery products due to their convenience, palatability irrespective of economic status. Traditionally, they comprise of refined flour, sugar, and fat, thus lacking nutrient density and dietary fibre. The shift in food habits with increased intake of empty calorie foods, coupled with a sedentary lifestyle is bringing nutrition transition resulting in rising incidences of lifestyle disorders adding to the burden of malnutrition. Understanding these health complications, consumers are demanding palatable yet healthy snack alternatives. Thus, paving way for development of designer products that are fortified with health promoting ingredients to enhance the value of normally consumed foods by the population. Bakery products being liked by all can be a suitable medium for value addition to improve their nutritive value. Bran is a by-product of primary processing such as dehulling, milling and debranning of grains. It has rich nutrient, antioxidants and phytonutrients profile but is discarded or remains underutilized. Previous attempts have highlighted the use of some cereal brans in value addition of bakery products, but minimal studies are available on utilization of minor millet brans. Thus, the present study aimed to reformulate buns and muffins with the addition of minor millet brans to enhance their nutrient profile.

In the present study, bran was obtained by dehulling minor millet viz. kodo, proso, foxtail and barnyard. Per cent bran recovery (%) for all the grains after dehulling ranged from 12.35 to 17.93 per cent with kodo millet having highest percent bran recovery and reduction in grain size. Bran samples were immediately stabilized using different treatments for lipase inactivation and improving their storage. Among all treatments microwave heating (900 W at 2.5min) was found to be best in controlling the free fatty acid and moisture during 15 days storage. Nutrient analysis of stabilised bran indicated that amongst all the brans, protein (13.04 g/100 g), zinc (5.59 mg/100 g) and potassium (630.83 mg/100 g) content was highest in proso bran (PSM), whereas foxtail bran (FSM) had highest fat (9.87 g/100 g), iron (65.58 mg/100 g) and calcium (94.63 mg/100 g). Total dietary fiber (TDF) was highest in kodo bran (61.52 %) (KSM), and sodium content was highest in barnyard bran (BSM). Phytonutrients like total phenol (449.27mg GAE/100 g), flavonoids (22.37 µg RE/ g) and phytic acid (630 mg/100g) were significantly higher in KSM thus, enhancing its antioxidant activity compared to other brans in terms of DPPH, TBARS and FARP assay. Calculation of phytate minerals molar ratios showed that PSM and FSM had phytate/iron molar ratio well below critical levels. Phytate/zinc molar ratio was within critical level for PSM, BSM and FSM, whereas only BSM had phytate/calcium ratio within critical limits. Functional properties of bran revealed water (197.76 – 265.48 ml/100 g) and oil (162.62 - 258.19 g/100 g) absorption capacity was nearer to that of other cereal brans, but they had no foaming capacity.

Further, for product formulations, bran was blended in different concentrations (0, 10, 15, 20, 25 and 30%) in refined wheat flour. Functional properties of flour combinations revealed that irrespective of brans, water absorption capacity increased but, water solubility, oil absorption and foaming capacity decreased as percent bran increased. Amongst these blended flours, it was discovered that irrespective of brans, muffins with 30 per cent and buns with 20 per cent bran were acceptable with a sensory score above 7. After finalising the bran concentration (%), amongst all four bran, bun with foxtail (20%) and muffins with kodo (30%) bran was selected for nutritional, shelf life and supplementation studies. Bran enriched bun (FB20) and muffin (KM30) had significantly (p,0.05) higher nutritional, antioxidant and phytonutrient profile compared to their respective controls. Percent increase in TDF was 272 per cent, 268.92 per cent in KM30 and FB20 respectively. FB20 and KM30 had better iron (13.83, 7.86 mg/100 g), calcium (23.90, 40.68 mg/100 g) and zinc (4.53, 0.85 mg/100 g) content compared to their respective controls. Shelf life of buns and muffins was 3 and 7 days respectively. Glycaemic index (GI) and Glycaemic load (GL) of both buns (57.71, 19.32), muffins (56.42, 14.85) fell under medium category.

Supplementation study (45 days) in two groups (muffin, n=14) (bun, n=15) with adolescent subjects revealed that blood glucose levels reduced significantly in bun group, but reduction was not significant in muffin group. There were no significant changes in total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), TC to HDL ratio. Analysis of stress profile indicated an insignificant decrease in lipid peroxidation and a significant increase in glutathione reductase, whereas no significant change in RBC catalase values in both groups. Though there was a reduction in glucose levels, lipid peroxidation, and an increase in glutathione reductase findings suggest that longer duration, controlled trials are essential for establishing concrete results on blood profile. Thus, present study concluded that minor millet brans can be used as a functional ingredient for enhancing the nutritive value of commercially available empty calorie snacks.

Chapter I

INTRODUCTION

Consumption trend has globally witnessed a lot of change over the past few decades and so has the food processing industry. These changes are mainly triggered by industrialization and urbanization. Increase is noted in consumption of high calorie foods, meat, processed foods, fast-foods, bakery products lacking in fiber and other essential nutrients coupled with sedentary life style (Kearney, 2010; Joseph *et al.* 2015; Küster and Vila, 2017 and Fuhrman, 2018). Refined foods especially the bakery products such as biscuits, cakes, muffins, breads, buns, pizza and burgers are replacing whole grain foods decreasing the daily intake of fiber. Consumption of these bakery products has drastically spiked and are becoming popular in all age groups across the world (Lebesi and Tzia, 2011; Caleja *et al.* 2017; Gul *et al.* 2017 and Safdar, 2019). Bakery products are preferred by consumer's as they are ready to eat convenient foods and easily available take away options for snack time. The changes in snacking pattern and overall dietary habits have also led to adverse transition in nutrition and health causing increased vulnerability and incidences of non-communicable life style disorders like obesity, diabetes, hyper tension and cardiovascular disorders (Safdar, 2019 and Akbarpou *et al.* 2019). Increased snacking habits on these foods has also posed threat of double burden with under nutrition on one hand and obesity on other (Kearney, 2010). Research also suggests that high intake of refined carbohydrates, commercially baked goods, fats, processed foods, fast food and sweets might even cause mental illness (Fuhrman, 2018).

Understanding these adverse effects on health, consumers are now becoming more aware about nutrition and health impacts of their food choices, thus are demanding nutritious yet palatable and delicious snacks. This challenges the food industries to upgrade themselves and formulate nutrient rich and tasty snack substitutes by bringing designer or fortified foods in market (Jisha *et al.* 2010; Lebesi and Tzia, 2011 and Küster and Vila, 2017). Rajasekaran and Kalaivani (2013) state that designer foods are prepared, fortified or enriched with functional ingredients providing additional health benefits along with the nutrients already present. With the changes in life style; dietary fiber, phytochemicals and antioxidants are gaining more attention in functional food market due to their potential health benefits, and researchers are trying to harness these functional ingredients from natural sources as consumers are demanding minimally processed foods (Caleja *et al.* 2017).

Bakery products are mainly calorie dense foods usually prepared with ingredients *i.e.*, refined flour, sugar and fat thus they lack many essential nutrients. Being a popular snack among all age groups scientists have conducted numerous studies on improving their nutritional, fiber and antioxidant content. Researchers have reformulated the breads, biscuits, muffins, buns etc. with various sources of dietary fiber extracted from vegetable pomace, fruits peels, seeds and cereal brans (Ajila *et al.* 2008; Kohajdova *et al.* 2012; Ogunbusola *et al.* 2012; Patel, 2015; Mildner-Szkudlarz *et al.* 2016 and Heo *et al.* 2019). Among all these sources extensive research is being conducted on use of cereal bran considering the dietary fiber, vitamins, minerals and phytonutrients packed in bran that provide health benefits and reduce risk of lifestyle disorders upon consumption of whole grains (Vitaglione *et al.* 2008 and Lattimer and Haub, 2010).

Bran is a hard-outer layer of cereals and millets consisting of aleurone layer, testa and pericarp that is usually discarded during primary processing such as decortication, dehusking, debranning, dehulling, polishing and milling (Saleh *et al.* 2013; Bagchi *et al.* 2014 and Kalpanadevi *et al.* 2018). These primary processes are vital for grains to improve their edibility, sensory palatability and also to ease further processing or cooking (Saleh *et al.* 2013 and Patel, 2015). Processing of cereals is easier compared to millets given the availability of industrial level processing equipment and grain size. Whereas primary processing of millets especially minor millets is tiresome due to their tiny grain size and lack of suitable dehulling equipment thus a major part of grain is lost in form of bran and bran rich fraction also causing loss of major share of nutrients (Gulia *et al.* 2007; Suma and Urooj, 2012 and Saleh *et al.* 2013). Reduction is reported in the nutrients, dietary fiber, tannins, flavonoids, polyphenol content and antioxidant potential of whole grain, when the bran is removed during refining, indicating that bran is a good source of these components (Yu *et al.* 2013; Devi *et al.* 2014; Goudar and Sathisha, 2016; Ahmad *et al.* 2018 and Bora *et al.* 2018).

Studies on nutritional profiling on bran suggest that they are rich source of dietary fiber, minerals and phytonutrients like polyphenols, tannins and phytates. These phytonutrients present in bran contributes to its antioxidant capacity. Thus, cereal bran (wheat, rice and oat) and millet brans (sorghum, finger, pearl, little, kodo, proso, foxtail and barnyard) are reported to exhibit excellent antioxidant capacity (Dar and Sharma, 2011; Sridevi *et al.* 2011 and Bisoi *et al.* 2012). Previous studies also provide evidences that supplementation of rice, wheat, oat and barley bran may have hypoglycaemic and hypolipidemic effect as carbohydrate present is complex in nature like β-glucan, arabinoxylan, cellulose, pectin, gums (Qureshi *et al.* 2001; Liu *et al.* 2014; Abulnaja and El Rabey, 2015; Steinert *et al.* 2016 and Sahrir *et al.* 2017).

Amount of bran obtained, varies from grain to grain depending on its structure and processing factors like equipment used, time taken, degree of dehulling or milling. Considering the total grain weight, 13–19 per cent of bran is obtained from wheat (Onipe *et al.* 2015) and 5 – 8 per cent from rice (Arendt and Zannini, 2013a). Considering the structure of minor millets husk and bran content varies from 13.5 per cent husk and 1.5 – 2 per cent bran in foxtail millet, up to 37 per cent in kodo millet and 23 per cent barnyard millet (Pawar and Machewad, 2006; Taylor and Emmambux, 2008; Dharmaraj *et al.* 2016; Sharma and Niranjan, 2018 and Serna-Saldivar and Espinosa-Ramırez, 2019). Primary processes like milling, dehulling, dehusking, debranning for small millets, approximately results in removal of 16 – 17 per cent bran. In India, the production of jowar in 2015-16 was 4.24 million tonnes (MT), bajra 8.09 MT, ragi 1.82 MT and small millets 0.39 MT (Directorate of Economics and Statistics, 2018). Considering only the production data of small millets approximately 0.062 to 0.066 MT bran is generated after dehulling. This bran obtained as by-product is either wasted or also used as animal feed (Liang *et al.* 2010).

Despite the nutritional benefits one of the main reasons behind disposal of bran is its shorter shelf life due to production of free fatty acids (FFA) (Sharma *et al.* 2014). Thus, after obtaining bran it needs to be stabilised immediately. Successful stabilization of cereal brans using different processes like autoclaving, hot air oven heating, microwave heating, ohmic heating, extrusion, chemical treatments and bioprocessing is reported by various authors but limited reports are available on stabilization of minor millet brans (Akhter *et al.* 2015; Bhosale and Vijayalakshmi, 2015; Loypimai *et al.* 2015; Nazni and Karuna, 2016 and Lv *et al.* 2018). Stabilization helps in inactivating lipase activity thereby reducing the FFA production and improving the shelf life. Stabilized bran thus can be further used in food processing. Another challenge for supplementing bran in bakery products is its negative effect on texture. But various processes like heating, soaking, bioprocessing, reducing particle size (microparticulation or micronisation) or fermentation can help in reducing the negative effect (Kim *et al.* 2013a and Ahmad *et al.* 2018). According to previous studies cookies or biscuits with rice and wheat bran incorporation up to 30 per cent were acceptable (Suma and Nandini, 2015). Similarly, enzymatically treated sorghum and millet bran incorporation in breads up to 20 per cent showed better acceptance (Ahmad *et al.* 2018). Replacement of wheat flour with 10 per cent cereal bran and 15 per cent of barnyard millet bran was acceptable for muffins (Lebesi and Tzia, 2011 and Nazni and Karuna, 2016). The literature survey thus depicted that limited studies are available on millet bran incorporation in bakery products. It is also evident that reformulating bran enriched bakery products without affecting sensory parameters is a challenge for food processing industry (Coda *et al.* 2015). Thus, there is need to conduct research on developing designer buns and muffins using minor millet bran without affecting sensorial acceptance by consumers. Although plentiful research work is done on stabilization, nutrient profiling and utilization of cereal bran for enriching bakery products limited work is available of millet bran (Suma and Urooj, 2012). Even though many studies have been conducted on supplementation of cereal bran, scanty data is available on supplementation of minor millet bran to enrich products and its effect on blood glucose, lipid or stress profile. Considering all these factors the present study entitled "Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential" was planned to examine the minor millet brans for their nutritional composition and health benefits. The work was planned and carried out systematically under the following objectives:

1. To stabilize selected millet brans and analyze its nutrients, anti-nutrients and antioxidant potential
2. To formulate designer products incorporating the millet bran and conduct sensory evaluation
3. To assess the physico-chemical characteristics and nutrient composition of the accepted products
4. To conduct shelf-life study for the accepted products
5. To study the therapeutic potential of the developed products

Chapter II

REVIEW OF LITERATURE

Dehulling is done traditionally for coarse grains and millets to improve its edibility, yielding by-product bran – a hard-outer layer rich in bioactive compounds and phytochemicals which is generally discarded or underutilised. Presently attempts are made to utilise several cereal brans like rice bran, oat bran and wheat bran as healthy human food source through products like rice bran oil, incorporating bran in various bakery products and snacks providing fibre rich products etc. Process of minor millet dehulling is cumbersome as the grain size is small and lack of suitable dehulling equipment thus, a major part of grain gets dehulled. Millet brans are obtained after milling and dehulling millets which usually get discarded. The nutritional content and therapeutic potential of millet bran make them a promising source of human food which can be utilised for value addition of energy-dense (empty calories food) or processed foods. Tapping these underutilised millet brans obtained after dehulling kodo millet (*Paspalum scrobiculatum*), proso millet *(Panicum miliaceum)*, barnyard millet *(Echinochloa esculenta)* and foxtail millet (*Setaria italica)*, to develop designer foods was the aim of the present study. The literature review suggests that there is limited research conducted on stabilisation, nutritional analysis, product development, storage studies and clinical trials for minor millet bran. Thus, in this chapter along with minor millet brans, literature available on the major millets and cereal brans is also reviewed and presented under appropriate sections.

2.1 Minor millets and their nutritional profile

2.1.1 Foxtail millet

2.1.2 Proso millet

2.1.3 Kodo millet

2.1.4 Barnyard millet

2.2 Processing millets and cereals to obtain bran

2.3 Nutrient, anti-nutrient and antioxidant quality of millet and cereal bran

2.4 Therapeutic potential of millet and cereal bran

2.5 Stabilisation of millet and cereal bran

2.6 Formulation, sensory evaluation and physicochemical properties of designer products incorporating millet and cereal bran

2.6.1 Cookies and biscuits

2.6.2 Pasta and extruded products

2.6.3 Bread and buns

2.6.4 Muffin and cupcakes

2.6.5 Products prepared using other sources of fiber

2.7 Nutritional composition, glycaemic index, glycaemic load and therapeutic potential of designer products incorporated with millet and cereal bran

2.8 Shelf life of designer products incorporated with millet and cereal bran

2.1 Minor millet and their nutritional profile

Millets are small seeds belonging to *Gramineae* (grass) family having a variety of species with some cultivated by farmers from Asia, Africa and some grown as wild grasses/weeds (Chandi and Annor, 2016). Millets are referred to as 'Nutri-cereals' and various studies make it evident that millets are nutritious, high in micronutrient, dietary fibre, antioxidants, phytonutrients and having low glycaemic index (GI) thus offering various health benefits (Saleh *et al.* 2013; Devi *et al.* 2014; Mishra *et al.* 2014; Upadhyaya *et al.* 2016 and Revankar *et al.* 2018). Despite being nutritional, some of these millets are neglected or underutilised. In India production of jowar in 2015-16 accounted to 4.24 million tonnes (MT), bajra 8.09 MT, ragi 1.82 MT, small millets 0.39 MT and other Nutri/coarse cereals production 38.52 MT (Directorate of Economics and Statistics, 2018).

Based on their size millets are classified as major and minor millets. Major millets include sorghum and pearl millet whereas, minor millets include finger millet, foxtail millet, little millet, kodo millet, barnyard millet, proso millet, and browntop millet (Bhat *et al.* 2018). They are botanically classified into caryopses and utricles. The pericarp is strongly attached to the seed in caryopses whereas in utricles seed coat is attached to pericarp only at one point making it easy for removal. Kodo millet is caryopsis-type while proso, foxtail and barnyard are utricle type (Taylor and Duodu, 2015; Taylor and Kruger, 2016 and DayakarRao *et al.* 2018). In another study kodo, foxtail and barnyard were classified under caryopsis-type whereas proso under utricle type (Serna-Saldivar and Espinosa-Ramırez, 2019). All these minor millet also have identical basic structure like other millet grains consisting of pericarp, seed coat/testa, aleurone layer, endosperm and germ that is represented in the Fig 2.1. Though the general millet structure is similar in all millet grains significant variation is noted in fine structure (ratio of endopserm to germ, thickness of pericarp) amidst different varieties (Akanbi *et al.* 2019)

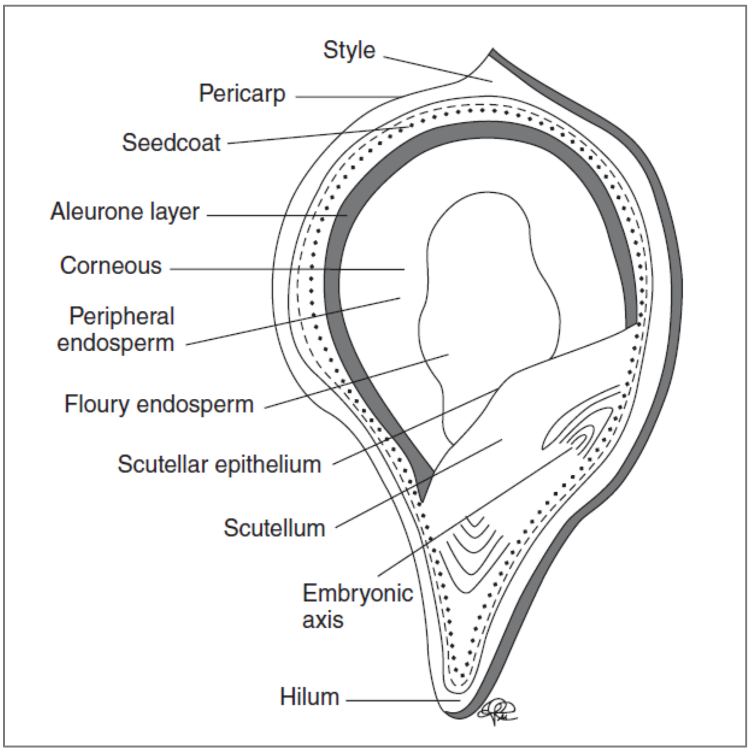


Fig 2.1 General structure of millet grain (pearl millet)

(Source: Reprinted from Arendt and Zannini (2013b), with permission from Elsevier)

2.1.1 Foxtail millet

Foxtail millet (*Setaria italica)* is one of the oldest cereals originated in China and cultivated in China, India, Africa, southern United States (Liang *et al.* 2010 and Heuzé *et al.* 2015). Sowing time for foxtail in karif season is June to July and for rabi season is September to October. Important varieties of foxtail millet grown in India are Pant setaria-4, TNAU-43, HMT-100-1, SIA-326, PS-4, K-2, K-3 and Krishna devaraya (Directorate of Millets Development, 2017a). Approximately after 75 – 90 days of plantation foxtail crop is ready for harvesting and it can grow up to 2 – 5 feet tall (Sharma and Niranjan 2018). A major portion of the grain is husk (13.5 %) and 1.5 – 2 per cent constitutes bran and germ covering the endosperm (Pawar and Machewad 2006; Sharma and Niranjan 2018 and Dharmaraj *et al.* 2016). Foxtail millet is a good source of protein, fat, complex carbohydrates, fibre, minerals also antioxidants and phytochemicals. It contains 12.3 per cent protein 4.3 per cent fat, 60.9 per cent carbohydrates, 14 g dietary fiber, 3.3 per cent mineral matter, 31 g calcium and 5 mg iron (Habiyaremye *et al.* 2017). Traditionally the grains are processed for further utilisation and can be cooked similarly as rice, finds application in bread, cake, biscuits, noodles, pudding (Heuzé *et al.* 2015), porridges, wine and nutrition powder (Liu *et al.* 2012). With increasing awareness regarding its nutritional value foxtail millet has entered into daily consumption of many. The grain, as well as husk and bran obtained from processing such as dehulling, also finds application as animal feed (Heuzé *et al.* 2015). Fig 2.2 represents foxtail millet grain and its spikelet.

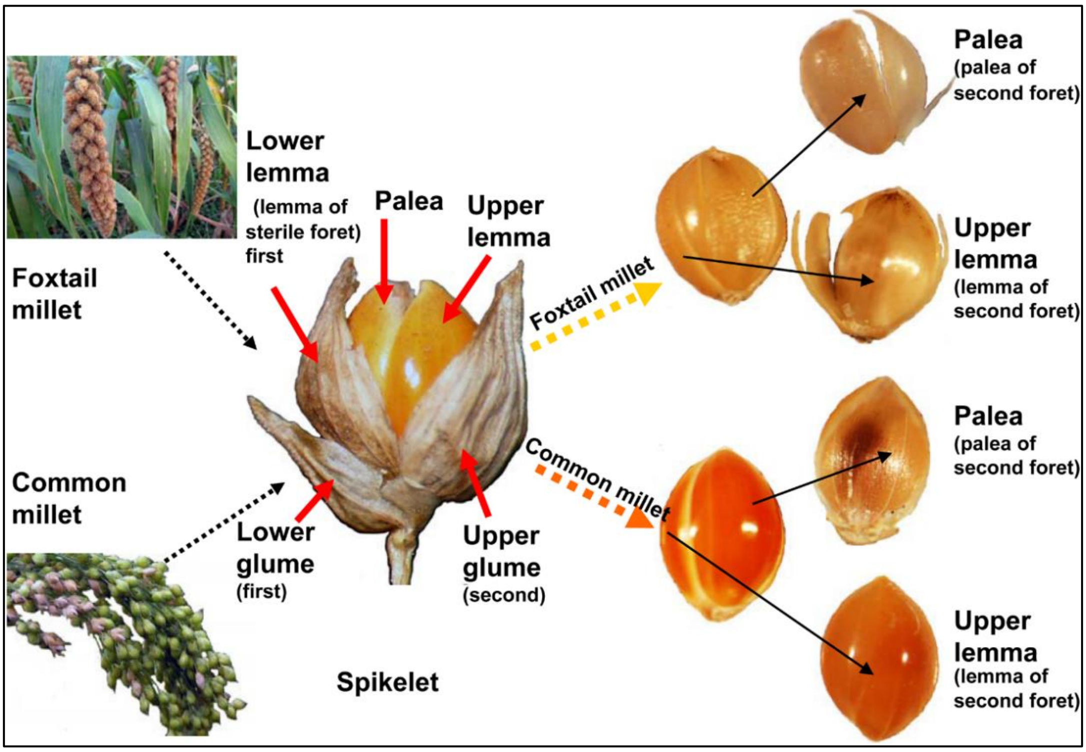


Fig 2.2 Spikelet and grain of foxtail and proso millet

(Source: Reprinted from Lu *et al.* 2009)

2.1.2 Proso millet

Proso millet *(Panicum miliaceum)* is grown in some parts of Asia, Australia, North America, Europe, Korea and Africa. It is also known as cheena, broomcorn millet, common millet, and hog millet (Azad *et al.* 2019). Fig 2.2 represents proso millet grain and its spikelet. It is used as bird, animal feed and also human food (Rajput *et al.* 2014 and Upadhyaya *et al.* 2016). Important varities of proso millet grown in India are TNAU-151, TNAU-164, GPUP-8, Pratap chena-1, Bhawana, Nagarjuna, PRC-1 and K-1

(Directorate of Millets Development, 2017b). Proso millet is warm climate or summer crop and it even grows well in non-irrigated areas. To grow completely it takes 60 – 100 days and becomes 30 – 100 cm tall. Proso millet contains (per 100 g) about 12.5 per cent protein, 3.1 per cent fat, 70.4 per cent carbohydrates, 14.2 per cent dietary fiber, and about 1.9 per cent mineral matter (Habiyaremye *et al.* 2017). It is also reported to have good antioxidant potential due to the presence of phenolic compounds (Bora *et al.* 2018 and Fei *et al.* 2018). It is reported that proso millet husk has smooth texture and the endosperm to germ ratio is 12 : 1 or 11 : 1 as it has small embryo (Arendt and Zannini, 2013b). Proso millet is consumed in the form of rice, and it can be used for value addition of various products like *idli, wada, payasam* etc.

2.1.3 Kodo millet

Kodo millet (*Paspalum scrobiculatum*) can grow, survive in harshest conditions, has a good nutritional profile but remains understudied and underutilised (Fig 2.3). It is grown in various parts of India including Maharashtra, Madhya Pradesh and Uttar Pradesh (Yadav *et al.* 2013). Important varities of kodo millet grown in India are JK-13, JK-48, GK-2, Vamban, IPS 147-1, JK-62, JK-76, GPUK-3 and Kherapa (Directorate of Millets Development, 2017c). Kodo millet approximately takes 4 – 6 months to grow and be ready for harvesting (Taylor and Emmambux, 2008). Kodo millet has a greater portion of bran and husk contributing almost 37 per cent (Malleshi and Hadimani, 1994; Taylor and Emmambux, 2008 and Serna-Saldivar and Espinosa-Ramırez, 2019) making it rich in crude fibre and dietary fibre contributing to the hypoglycaemic effect. Kodo millet (per 100 g) is reported to have 8.3 – 9.8 g protein, 1.4 – 3.6 g fat, 3.3 g ash, 5.2 g crude fibre, 15 g dietary fiber, 65 – 66.6 g carbohydrate, 353 Kcal energy, 27 – 35 mg calcium, 1.7 – 12 mg iron, 0.15 mg thiamine, 0.09 mg riboflavin and 2.0 mg niacin (Saleh *et al.* 2013 and Habiyaremye *et al.* 2017). Whole grain, dehulled grains, grain fraction, husk or bran of kodo millet have excellent antioxidant potential (Chandrasekara and Shahidi, 2011). Kodo millet can be used both as animal feed and also human food to prepare products like rice, bread, biscuits, cakes, cookies, *idli, upma, chapati, pongal, laddu* etc., (Yadav *et al.* 2013, Karuppasamy *et al.* 2013 and Deshpande *et al.* 2015).

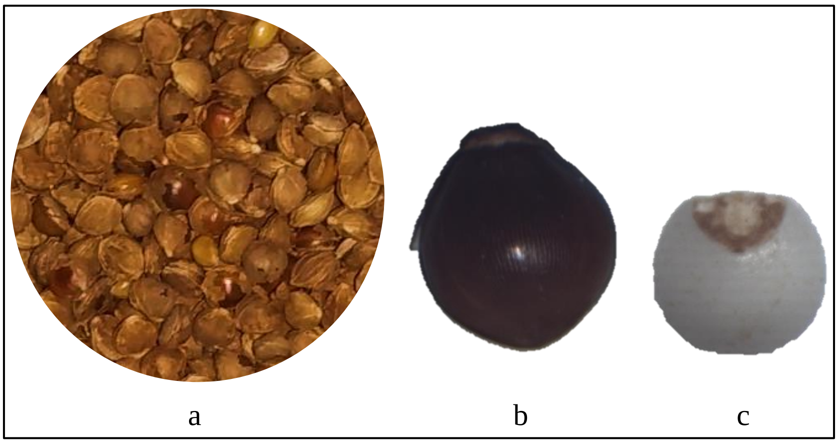


Fig 2.3 Kodo millet grains (a) whole (b) and dehulled (c)

(Source: Photo clicked by author)

2.1.4 Barnyard millet

Barnyard millets have two different species Japanese barnyard millet (*Echinochloa esculenta*) being cultivated in East Asia, Japan, Korea, China and Indian Barnyard millet (*Echinochloa frumentacea*) cultivated widely in Indian subcontinent and china (Taylor, 2019). Important varieties of barnyard millet cultivated in India are VL-Madira-181, VL-Madira-172, Pratap sanwa-1, kancahn, VLM-172 and VL-Madira-2007 (Directorate of Millets Development, 2017d). Barnyard millet (per 100 g) is reported to have 6.2 g protein, 65.5 g CHO, 2.2 g fat, 13.7 g dietary fiber, 4.4 g mineral matter, 11g calcium and 15 mg iron (Habiyaremye *et al.* 2017). The bran, husk or glumes contribute to 23 per cent of the total weight of the grain (Serna-Saldivar and Espinosa-Ramırez, 2019). Barnyard millet is a fair source of protein, fat, good source of fibre, minerals and also antioxidants (Amadou *et al.* 2013; Ugare *et al.* 2014; Verma *et al.* 2015 and Serna-Saldivar and Espinosa-Ramırez, 2019). It can be consumed as rice, and flour is used in the preparation of *chapati*, biscuits, cakes, etc., The millet exhibits hypoglycaemic properties making it beneficial for diabetes patients (Ugare *et al.* 2014) also due to its antioxidant potential barnyard millet can fight or prevent various diseases conditions associated with free radicals production (Amadou *et al.* 2013).

2.2 Primary processing of millets and cereals

Cereals and millets are processed before consumption using many processing techniques like dehulling, polishing, winnowing, milling, heating, baking, microwave heating, autoclaving, steaming, boiling, fermentation, germination, malting, roasting, flaking, and grinding mainly to improve its edibility, palatability and shelf life (Saleh *et al.* 2013 and Kadiri, 2017).

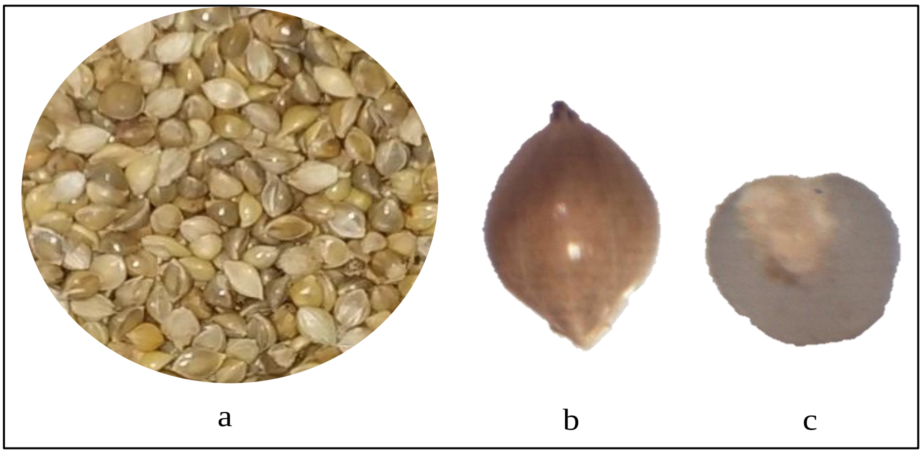


Fig 2.4 Barnyard millet grains (a) whole (b) and dehulled (c)

(Source: Photo clicked by author)

Dehusking, dehulling and polishing are primary processing mainly done to remove the husk, hull and brans thus improving cooking quality of grain. Milling is the process where the grain is ground in flour or meal, with a focus on husk and bran removal. It is one of the important and necessary steps to make the grain accessible for further utilization. Most food production requires grains to dehull and mill, yielding a by-product, i.e., bran – outer layers of the grain. This primary processing mainly reduces the grain size, some nutrients like fiber, minerals, antioxidants and phytonutrient content of grains but also increases digestibility and availability of other nutrients especially those, that are bound to these antinutritional factors (Oghbaei and Prakash, 2016).

Millet brans are a by-product of millet processing (Devittori *et al.* 2000 and Taylor, 2016). Given the grain sizes and lack of specifically fabricated dehullers, various authors have reported the use of rice dehuller, miller, barley pearlers or abrasive dehullers etc., for dehulling and processing minor millets grains (Chandi and Annor, 2016). These primary processings like decortication, dehulling are also done traditionally by manual hand pounding method. Three different techniques, like abrasive decortication, rubbing technique or metal friction decortication, are used for dehulling minor millets. Pulversizing minor millets can be done by hammer mills, burr mills, and plate or disk mills (Chandi and Annor, 2016). A considerably greater portion of grain i.e., bran, bran-rich fraction and husk is lost in processing given its small size, thus resulting in greater loss of nutrients (Gulia *et al.* 2007 and Suma and Urooj, 2012). Bran/hull obtained by using these processing techniques from any variety of millets such as proso, foxtail, pearl millet, jowar etc. are utilised as animal feed due to it nutritional potential (Tran, 2015). Millet bran is not collected commercially like rice or wheat bran and is usually discarded as waste by-product.

2.3 Nutrient, anti-nutrient and antioxidant quality of millet and cereal bran

Millets are referred to as 'Nutri cereals', and they confer lots of health benefits. Cereals, millets and their bran fractions obtained as a result of milling and dehulling are a rich source of protein, fat, fibre, dietary fibre, ash, minerals, vitamins, phenolic compounds, flavonoids, anthocyanins, tannins, etc. Antioxidant activity is due to the various phytochemicals present in the plant. These phytochemicals play a vital role in preventing body against reactive oxygen species (ROS). These antioxidants required to prevent and control degenerative diseases are found in higher proportions in bran and bran-rich fractions viz., aleurone layer, testa, and pericarp of cereal grains. Thus, processing the cereals (dehulling, milling, debranning, dehusking etc.) results in the reduction of polyphenol content as some amount is lost through the hull, bran and bran fractions which are discarded (Dykes and Rooney, 2007; Suma and Urooj, 2014; Devi *et al.* 2014; Devisetti *et al.* 2014 and Kadiri, 2017). Summary of the nutrient composition of minor millet bran (kodo, proso foxtail and barnyard) compared to rice, wheat and sorghum bran is presented in Table 2.1

Different varieties of proso millet and bran were found to contain different levels of lipid. The free and bound lipids found in red varieties of proso millet bran ranged from 3.45 – 4.29 per cent and 0.30 – 0.70 per cent respectively, whereas white varieties had 3.95 – 6.84 per cent (free) and 0.33 – 0.49 per cent (bound). The total lipid content in bran of red and white varieties ranged from 4.14 – 7.27 per cent. Linoleic, oleic and palmitic acid represents 90 per cent of fatty acid in proso millet flour and bran (Lorenz and Hwang 1986).

Proso bran obtained from Sonček variety treated with ultrasound treatment (80 % amplitude for 12.5 min) increased the antioxidant activity by 15 per cent (FRAP test) and 16 per cent (TPC). Increase in water and ethanol soluble Dietary fibre (DF) content was noted when ultrasound treatemetn was given at 100 per cent amplitude for 20 min (Mustač *et al.* 2019).

Micronisation of proso bran also resulted in increased antioxidant value measured by using FRAP and ABTS assay. It was also noted that total soluble fiber increase in cryo-ground (8 – 12 min) bran samples compared to control (Mustač *et al.* 2020).

Foxtail millet bran obtained after milling had higher phenol content compared to whole or pearled grain. In foxtail bran phenol content (mg/100 g) was highest (510.53 ± 50.34), followed by whole grain (132.76 ± 8.66) and least content was in pearled grain

(104.00 ± 0.00). Zinc, copper and phytic acid showed no significant difference in whole grain or millet fractions (Sridevi, 2007).

Foxtail millet bran was reported to have 9.39 ± 0.17 per cent crude oil, 12.48 ± 0.41 per cent crude protein, 51.69 ± 2.14 per cent crude fibre, 7.50 ± 0.18 per cent ash and 8.29 ± 0.16 per cent of moisture. Oil extracted from foxtail millet bran showed tocopherol content of 64.83 ± 0.83 mg/100 g oil. It mainly consisted of γ-tocopherol (48.79 ± 0.46 mg/100 g oil) and α-tocopherol (15.53 ± 0.31 mg/100 g oil). Foxtail millet bran oil also contained linolenic (66.5 %) and oleic acid (13.0 %) and saturated fatty acids like palmitic acid and stearic acid (Liang *et al.* 2010).

Proximate analysis of full fat foxtail millet bran (FMB) and DFMB showed that fat removal resulted in increased ash (7.78 ± 0.20 % to 10.32 ± 019 %) and protein (12.93 ± 0.21 % to 14.82 ± 0.57 %) content respectively. Defatting reduced fat content from 9.63 ± 0.15 to 1.85 ± 0.08 per cent respectively. Crude fibre content of FMB was 42.56 per cent indicating high fibre content. Composition of FMB oil demonstrated that it had 87 per cent of monounsaturated fatty acids, 4 per cent polyunsaturated fatty acids and 9 per cent saturated fatty acids (Amadou *et al.* 2011).

Type of extraction plays a critical role in detecting phytochemicals. Methanol, ethanol and water extracts of whole flour and bran-rich fractions of foxtail millet bran showed the presence of terpenoids and tannins in all extracts; flavonoids, alkaloids, phenolics and reducing sugars were found only in methanol and aqueous. Saponin was detected only in the aqueous extract. In bran-rich fractions, the methanolic extract had the highest radical scavenging activity (51.8 %) than alcohol (42.90 %) and water (33.60 %) extracts. Antioxidant activity was highest in bran-rich fractions than whole flour. The methanolic extract of whole flour and bran-rich fractions showed significantly higher reducing power ($p<0.05$) compared to other extracts (Suma and Urooj, 2012).

Similarly ethanol:water (3:7 v/v; 1:1 v/v and 7:3 v/v) extracts of defatted foxtail millet bran (DFMB) had good phenol content ranging from 29.39 ± 1.36 to 21.49 ± 3.26 GAE/100g extract. Total phenolic content was highest in DFMB ethanol: water (1:1 v/v) extract (29.39±1.36 GAE/100 g). DFMB thus demonstrated good antioxidant potential reducing power, scavenging abilities on DPPH, ABTS and superoxide radicals (Amadou *et al.* 2011).

A total of 65 volatile compounds were identified in the extracts of brown millet (BM), milled millet (MM) and millet bran (MB) of foxtail millet (var. JIGU NO19). Eight compounds viz., hexanal, nonanal, (*E*)-2-nonenal, naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, hexadecenoic acid and 2-pentylfuran) were dominant in the MB materials. The proximate analysis of foxtail millet bran revealed that moisture, protein, fat, ash, and carbohydrate content were 9.07 ± 0.34 g/100 g, 6.78 ± 0.19 g/100 g, 5.65 ± 0.23 g/100 g, 2.15 ± 0.18 g/100 g and 76.35 ± 0.63 g/100 g respectively (Liu *et al.* 2012).

Whole grains, dehusked grains and husk of foxtail millet varieties (HMT-1-100, PSC-4, Sia-3126 and black) showed that TPC and TFC of husk was higher in husk compared to grains (whole and dehusked). Among all varieties black variety had high content of phenolics, thus high antioxidant property (% radical scavenging activity) *i.e.*, 69.41 per cent (whole), 64.17 per cent (dehusked) and 79.24 per cent (husk). Whereas Sia-3126 variety had least antioxidant property *i.e.*, 53.90 per cent (whole) 41.96 per cent (dehusked) and 60.71 per cent (husk). TPC for whole grains, dehusked grains and husk was ranging from 420.0 to 982.8, 240.3 to 505.3 and 547.6 to 1140.3 µg/g GAE respectively. TFC was ranging from 282.5 to 590.3 (whole), 230.1 to 432.4 (dehusked) and 352.4 to 840.2 (husk) µg/g RE for all samples (Goudar and Sathisha, 2016).

Anti-nutritional factors such as lipase activity, peroxidase activity, phytic acid, total phosphorous, and trypsin inhibitor decreased in chemically treated barnyard millet bran as compared to raw bran. The proximate content for bran soaked in hot water (125°C) for 15 minutes, filtered and mixed with 20 per cent solution of 1 per cent calcium hydroxide followed by filtering and drying at 50 °C had slightly higher protein (5.19 g), fibre (45.10 g), ash (23.10 g), starch (19.12 g) compared to raw bran and bran soaked in 20 per cent acetic acid, 0.07 per cent sodium bicarbonate solution followed by filtering and drying at 50 °C (Nazni and Karuna, 2016).

Kodo millet bran showed good amount of protein (4.92 %), carbohydrate content of 79.84 per cent, fat (2.83 %), total dietary fibre (48.42 %), ash (5.33 %), moisture content of 7.07 per cent and energy per 100 g was 364.5 Kcal (Sarma *et al.* 2017).

Hulls fraction of both kodo and pearl millet had 4 to 16 times higher TPC compared to their dehulled grain counterparts. Kodo and pearl millet hulls had TPC of 112 and 34.3 ferulic acid equiv µmol/g defatted meal respectively. Kodo millet hulls had 3 times more TPC than pearl millet hulls. ORAC (Trolox equiv µmol/g defatted meal) was 216 and 214 respectively for kodo and pearl millet hull whereas Hydroxyl Radical Scavenging Activity (ferulic acid equiv µmol/g defatted meal) was reported as 133 and 196 respectively, suggesting that hull fraction of kodo and pearl millets can serve as potential sources of health-promoting nutraceutical and functional food ingredients (Chandrasekara and Shahidi, 2011).

Analysis of the brans of kodo, proso, barnyard, finger millet, sorghum and wheat bran of tribal belt of Odisha had different levels of nutrients; moisture content was highest in finger millet bran, followed by wheat, proso, barnyard, sorghum and kodo millet bran. Crude fibre content was highest in barnyard bran (38.4 %) followed by kodo (34.24 %), proso (31.63 %) and lowest crude fibre was recorded in wheat bran (11.32 %). Wheat bran (12.19 %) had highest amount of protein and proso millet bran (4.13 %) had lowest content (Bisoi *et al.* 2012).

Antioxidants and phenolic content was significantly higher in foxtail, little, sorghum, brown ragi, white ragi and pearl bran than in flour or whole grain, whereas phytic acid did not differ significantly. Phenolic content of millet bran such as foxtail, little, sorghum, brown ragi, white ragi and pearl millet was reported as 570.53, 465.67, 309.40, 837.90, 235.00 and 483.53 mg/100 g respectively, while that of phytic content was 3.75, 2.05, 4.54, 4.71, 2.62 and 2.33 g/100 g respectively. Zinc content was high in bran of pearl millet (0.73 mg/100 g) followed by foxtail millet (0.72 mg/100 g) and brown ragi (0.66 mg/100 g). Brown ragi bran had higher copper content (0.64 mg/100 g) followed by foxtail millet (0.63 mg/100 g) and little millet (0.53 mg/100g) brans whereas sorghum bran had higher manganese (0.79 mg/100 g) content followed by durum wheat (0.74 mg/100 g) and little millet (0.70 mg/100 g) brans (Sridevi *et al.* 2011).

Amongst hydroxycinnamic acid bound arabinoxylans (HCA-AXs) extracted from brans of five Indian millet (finger, proso, kodo, barnyard and foxtail) varieties antioxidant activity evaluated using DPPH, FRAP and β-carotene linoleate emulsion assay showed that kodo millet bran had highest value with lowest EC_{50} value (567.5) in comparison to other millet. Thus, suggesting HCA-AXs, from millets can be exploited as free radical scavenging additives in functional foods (Bijalwan *et al.* 2016)

In similar way hulls obtained from different millet varieties of proso, foxtail and finger millet grown in Sri Lanka had higher amount of phenolic, flavonoid content and antioxidant activity compared to its whole or dehulled grain. Finger millet and its hull showed the highest phenolic content and antioxidant activities compared to other millets (Kumari *et al.* 2017a).

Milling of little millet yielded 27 per cent of hull. The phenol content of bran (465.67 ± 20.1) was higher compared to the whole grain (148.53 ± 6.31) and pearled grain

(78.63 ± 1.79). There was no significant difference in the phytic content, zinc, copper in whole grain or its milled fractions for both millets (Sridevi, 2007).

Different little millet varieties and its fractions were analysed for nutritional and antioxidant capacity. Bran showed higher amount of α-tocopherol, polyphenol and antioxidant activity compared to whole and decorticated grain. No significant difference was seen in phytic acid, iron, copper, zinc, manganese. The highest amount of α-tocopherol was observed in Chikkayagti -07 (4.80 mg/100 g) and lowest in Chadaval -35 (2.62 mg/100 g). Chikkayagatti -01 had highest polyphenol, antioxidant activity (235.40 mg/100 g; 33.89 %) whereas Chikkayagatti -07 had least (133.66 mg/100 g; 27.85 %). The polyphenol content in bran contributed to its antioxidant capacity (Kundgol *et al.* 2013).

Antioxidant content of bran rich fraction (BRF) of pearl millet varieties viz., Kalukombu (K) and Maharashtra Rabi Bajra (MRB) showed high antioxidant activity due to high tannin, phytic acid and flavonoid content. Tannins (31 g/100 g and 32 g/100 g) and phytic content (99 g/100 g and 66 g /100 g) was higher in BRF of both varieties than semi refined flour (21 and 19 g/100 g; 66 and 33 g/100 g) and whole flour (23 and 21 g/100 g; 78 and 57 g/100 g) respectively (Suma and Urooj, 2014).

Sorghum bran is reported to be good source of phytonutrients, fibre and antioxidants thus can be exploited as nutraceutical. Higher amount of phenols and tannins were reported in black and brown sorghum brans as compared to white sorghum bran and wheat bran. Dietary fibre ranged from 40 – 45 per cent in sorghum brans. The fibre and phytate content being high sorghum bran results in reduction of calorific value of product when used in product development (Dahlberg *et al.* 2004).

In sorghum and sorghum bran 3-deoxyanthocyanidins were detected which had antioxidant properties similar to anthocyanins and were stable to pH change. Compared to red (3.6 mg/g) and brown (3.6 mg/g) sorghum bran black sorghum bran (10.1 mg/g) had twice the amount of anthocyanins. Brans had 3-4 times higher levels of anthocyanins than whole grains. Thus, it was concluded that black sorghum had the highest potential to be used as source of natural food pigments (Awika *et al.* 2004).

Sorghum bran and freeze-dried crude phenolic extract (CPE) from bran have good antioxidant potential. The total phenols in the sorghum bran was recorded to be 10.12 mg (tannic acid equivalent) TAE/100 mg and CPE had 58.35 mg TAE/100 mg. Condensed tannins were 45.16 and 491.80 mg (catechin equivalent) CE / 100 mg in bran and CPE respectively. Antioxidant activity expressed in Trolox equivalent (TE) for both bran (0.60 mM TE/g) and CPE (4.60mM TE/g) was higher than whole grain (0.19 mM TE/g). Addition of CPE to sunflower oil resulted in reducing rate of increase in peroxide values (PV) and inhibited formation of primary and secondary oxidation products in oil compared to control oil without CPE thus increasing its oxidative stability during storage (Sikwese and Duodu, 2007).

Compared to conventional method, 96-well plate method was found to be robust and reproducible for assessing DPPH, flavonoid and phenolic content. Sorghum bran and flour analysed with conventional and 96-well plate method showed that DPPH scavenging activity, total flavonoid and phenolic content was higher in almost all bran samples than the flour Herald *et al.* (2012).

Phenol content of methanol extract from the bran of red sorghum was 38 mg/ml and higher amount was observed as 97 mg/ml in acidified methanol extract. Similarly total anthocyanin was higher in acidified methanol extract (242.7 mg/l) compared to methanol extract (98.59 mg/l). DPPH activity was also higher in acidified methanol extract of bran compared to methanol extract. This depicted that acidified methanol was better solvent for extraction than methanol (Kumari *et al.* 2013).

Xylanase-treated sorghum bran had highest total anthocyanin content (TAC) and lowest in fine sorghum bran (21.42 mg/kg), in millet variety (MB-87) bran also lowest value for TAC was reported in fine millet bran (21.47 mg/kg) and the highest by cellulase-treated millet bran (63.43 mg/kg). Condensed tannins were recorded lowest for fine sorghum bran and fine millet bran (172.38 mg and 171.80 mg/100 g respectively) while higher for coarse sorghum and millet bran (179.77 mg and 175.81 mg/100 g respectively). Enzymatic treatment favoured the extraction, detection of anthocyanins and also were effective in bringing down tannin content of brans (Ahmad *et al.* 2018).

Wheat bran had 80 per cent carbohydrates and more of pentoses than glucose. The ratio of arabinose to xylose in bran was 1.37. Wheat bran was also reported to have highest dietary fiber content than the whole wheat flour, sorghum and bajra (Nandini and Salimath, 2001).

Evaluation of antioxidant potential of cereal brans used by consumers as supplements of dietary fibres like oat brans (crunchy oat bran, oat bran alone, and oat breakfast cereal) and wheat brans (wheat bran alone, wheat bran powder, wheat bran with malt flavour, bran breakfast cereal, tablet of bran, and tablet of bran with cellulose) revealed that wheat bran and products prepared with wheat bran showed higher antioxidant activity compared to oat bran or products prepared with oat bran. Oat bran alone showed better antioxidant activity compared to oat bran products (Martinez-Tome *et al*. 2004).

Wheat bran fractions obtained after milling durum wheat showed that it had good amount of dietary fibre and had higher antioxidant potential owing to its phenolic content compared to semolina (whole). Antioxidant content of was highest in fraction C (layer closer to aleurone layer) compared to A (outer layer), B (intermediate layer), and two commercial bran products B&B50, B&B 70 (bran and brain 50, 70). B&B 70 had a total dietary fibre content of 61 per cent, while B&B 50 had 42 per cent (Esposito *et al.* 2005).

The nutritional content in bran varies based on grain to grain. Considering the importance of cereal dietary fibre wheat, oat, rye and maize can be used as functional ingredient. Total dietary fibre in wheat bran ranged from 36.5 to 52.4 g/100 g, rye bran has 35.8 g/100 g, oat bran consists of 18.1-25.2 g/100 g and maize bran has 86.7 g/100 g (Vitaglione *et al.* 2008).

Nutritional composition of wheat and oat bran revealed that oat bran had significantly higher protein (16.6 %) and fat (7.5 %) content compared to wheat bran (protein: 13.0% and fat:3.7%). TDF in wheat bran was 47.1 per cent and 16.5 per cent in oat bran (Talukder and Sharma, 2010).

In vitro anti-oxidant activity of xylo-oligosaccharides derived from cereal (rice, wheat and maize) and ragi millet brans revealed that coumaric acid was main bound phenols found in all brans. Coumaric acid was highest in rice bran and ragi bran. Other phenols identified were ferulic acid which was highest in ragi bran and lowest in rice bran. Syringic acid was also identified and ragi had highest content, but wheat had negligible content. The antioxidant activity by DPPH and FRAP method was exhibited highest in ragi bran and least in maize bran. In rice bran anti-oxidant activity by beta-carotene emulsion assay was highest followed by ragi bran. Overall results depicted that ragi bran had higher antioxidant as compared to other cereal brans (Veenashri and Muralikrishna, 2011).

Microparticulated wheat bran (MWB) with particle size 6.87(μm) had 362 Kcal/100 g calories, 8.0 g/100 g moisture, 5.4 g/100 g fat, 21.2 g/100 g protein, 8.2 g ash (g/100 g), 57.2 g/100 g carbohydrate, 14.9 g/100 g dietary fibre (Kim *et al.* 2013a).

Microfluidization significantly increased the antioxidant capacity of wheat bran. Wheat bran mixed with distilled water, passed through different interaction chambers viz., 200 mm (IC200) and 87 mm (IC87) in diameter of microfluidizer for 1-3 passes increased surface-reactive phenolic content (Wang *et al.* 2013).

Red, white and purple wheat bran obtained from various varieties evaluated for their proximate content revealed that purple bran had higher moisture, protein, and starch. Red bran had highest fibre (54.69 g/100 g) and ash (7.7 g/100 g) content (Fleischman *et al.* 2016).

Immature wheat bran (IWB) obtained from immature wheat harvested 10 days before the maturity period had higher content of ferulic acid (3091±32.9 µg/g) compared to MWB (1794±2.80 µg/g). Steamed IWB also had higher ferulic acid compared to MWB. p-Coumaric acid also was higher in IWB and steamed IWB compared to MWB. Thus antioxidant activity also depicted increase in IWB and steamed IWB compared to MWB (Kim *et al.* 2016).

Among Wheat bran (WB), oat bran (OB) and rice bran (RB), OB had highest protein (15.08 %), followed by RB (11.83 %) and WB (9.40 %), whereas RB had highest ash (6.72 %), fat (19.31 %) and dietary fibre (38.90 %) content. Carbohydrate content was noted highest in WB and least in RB (Kaur *et al.* 2017).

Solid-state fermentation of autoclaved wheat bran improved the nutritional properties. Total Dietary Fibre (TDF) and Soluble Dietary Fibre (SDF) content increased after fermentation, highest TDF amount (55.50 g/100 g) was observed in fermentation with lactic acid bacteria and least (52.02 g/100g) in autoclaved bran (AB). Autoclaving significantly increased (from 7.70 to 10.41 mg/g) the water extractable arabinoxylans (WEAX) in bran implying partial conversion of water unextractable arabinoxylans (WUAX) into WEAX. Fermentation also increased the WEAX content. Phytic acid degradation was significantly high in fermented brans and no degradation was seen in control bran (CB). LYFB (bran autoclaved at 120 °C for 20 min followed by fermentation with lactic acid bacteria and yeast at same time) had highest degradation of phytic acid (27.34%) while AB (autoclaved bran) had least degradation (6.26 %). Total phenols decreased after autoclaving the brans, but fermentation i.e. LFB (bran autoclaved at 120 °C for 20 min followed by fermentation with lactic acid bacteria) and LYFB increased the phenol content (Zhao *et al.* 2017).

Proximate composition of wheat bran was reported as moisture 7.2 ± 0.35 per cent, protein 25.59 ± 0.88 per cent, fat 4.62 ± 0.27 per cent, fibre 1.97 ± 0.21 per cent, ash 2.12 ± 0.12 per cent and carbohydrate 57.62 ± 0.44 per cent (Dhillon and Tanwar, 2018).

Nutritional content of rice bran stabilised at 110 °C for 20 minutes was reported to be 5.2 per cent moisture, 6.4 per cent protein, 4.7 per cent fat, 17.7 per cent fibre, and 17.7 per cent ash (Bagheri and Seyedein, 2011).

Rice brans showed to have maximum emulsifying capacities of 0.149 and 0.634; whereas maximum emulsion stability was 24.26 and 25.96 min. Foaming capacity was maximum of 98 per cent and 115 per cent; whereas foam stability was of 30.6 and 26.9 mL at 30 min. Rice bran proteins isolated from heat stabilised defatted rice bran had water absorption of 3.71 and 4.4 g/g and oil absorption of 4.24 and 5.13 g/g, making it a potential source of nutraceutical ingredient in food industry (Zhang *et al.* 2012).

Rice bran had mineral composition of calcium 2.26 per cent, Magnesium 0.88 per cent, potassium 0.23 per cent, sodium 36.131 mg/kg, iron 54.13 mg/kg, zinc 28.35 mg/kg, copper 1.566 mg/kg, manganese 71.12 mg/kg (Oluremi *et al.* 2013).

Ohmically stabilised rice bran had highest α-tocopherol (42.38 ± 0.53 μg/g) and γ-oryzanol (1190.1 ± 89.3 μg/g) and least α-tocopherol (11.12±0.48 μg/g) and γ-oryzanol (957.18 ± 53.7 μg/g) was noted in rice bran stabilised with steaming and oil extracted using immersion. Total antioxidant capacity was also higher in ohmically stabilised rice bran oil compared to steaming (Loypimai *et al*. 2015).

Bhosale and Vijayalakshmi (2015) compared nutritional composition of microwave stabilised rice bran (MSRB) and probiotic treated rice bran (PTRB). It was observed that the moisture, protein, fat content increased in PTRB, but decrease was observed in total dietary fibre, ash, calcium and zinc. The antioxidant capacity of MSRB was 65 Vit-C Eq. μg/g and PTRB had 70 Vit-C Eq. μg/g. It was noted that depending on nutritional and antioxidant capacity rice bran can be used as functional ingredient in food processing and availability of nutrients may increase due to different probiotic treatments.

Extrusion processing used for stabilising rice bran improved its functional properties. It enhanced the dietary fibre content, also improved water holding, foaming capacity but decreased oil holding capacity. Stabilisation also reduced the phytic acid content compared to unstabilised bran (Rafe *et al.* 2017).

2.4 Therapeutic potential of millet and cereal bran

Whole grain consumption is associated with reducing the risk of various aliments like cardiovascular diseases, type II diabetes, hypercholesterolemia, constipation, some cancers related to gastrointestinal tract etc. All these beneficial effects are due to the nutrients packed in whole grains like dietary fibre, vitamins, minerals, phytochemicals, phenolic compounds, mainly these components are concentrated in the bran (Dykes and Rooney 2007 and de Munter *et al.* 2007). Thus, millet and cereal brans possess good amount of fibre, minerals, phenolic compounds contributing to natural antioxidant potential which can find application in preventing and/or treating such ailments. Possible combination of fibre with antioxidant complex may be one of the reasons for therapeutic benefits of fibre.

Shan *et al.* (2014a) extracted and purified novel 35 kDa protein from foxtail millet bran. The results showed that the crude protein significantly suppressed colon cancer cell growth *in vitro* and xenografted tumours in nude mice by inducing cell cycle G1-phase arrest. Peroxidase derived from foxtail millet bran (FMBP) hampered migration of colon caner cells thus can be exploited as effective therapeutic agents for patients suffering with colon cancer (Shan *et al.* 2014b).

Foxtail millet bran oil (FMBO) showed high antioxidant activity in terms of FRAP and DPPH assay. It was also observed that it was effective in preventing hepatic injuries caused by ethanol in mice (Pang *et al.* 2014).

In another study bound polyphenols of inner shell (BPIS) from foxtail millet bran inhibited growth and induced apoptosis in human colorectal cancer HCT-116 cell and in HCT-116-bearing nude mice displaying anti-colon cancer functions through its pro-oxidant activity. BPIS increased ROS generation in HCT-116 cell inducing anti-proliferative effects at different concentrations (0, 0.57, 0.71, 0.88 mg/ml) in dose dependent manner (Shi *et al.* 2015).

Bound polyphenols of inner shell (BPIS) from foxtail millet bran also showed anti-inflammatory effects in controlling lipopolysaccharide (LPS)-induced HT-29 cells and nude mice. BPIS treatment of LPS-induced HT-29 cells downregulated IL-1β and IL-6 (pro-inflammatory molecules) and upregulated IL-10 (anti- inflammatory cytokines). Tumour tissue growth was also restrained by BPIS. Thus BPIS can be used as potential pro-oxidant agent against inflammation (Shi *et al.* 2017).

Table 2.1. Nutritional composition of minor millet brans compared to rice, wheat and sorghum bran

Nutrients	Brans						
	Rice[1]	Wheat[2]	Sorghum[3]	Foxtail[4]	Barnyard[5]	Kodo[6]	Proso[7]
Moisture (%)	5.2 – 13.3	7.2 – 12.7	6.13	6.10 – 9.07	6.79	5.53 – 7.07	7.32
Protein (%)	6.4 – 24.58	9.40-25.59	6.7	6.78 – 12.93	5.15 – 5.19	4.92 – 4.94	1.78-26.33
Fat (%)	4.7 – 20.31	2.95 – 5.4	4.6 – 4.9	5.65 – 9.63	4.04	2.83 – 3.36	1.92-8.96
Ash (%)	4.92 – 17.7	2.12 – 9.51	8.06	2.15 – 7.78	9.11 – 23.10	5.33 – 7.74	5.77-8.44
Crude fibre (%)	7.19 – 17.7	1.97 – 54.69	18.04	42.56 – 51.69	38.4 – 45.10	34.24	31.63
TDF (%)	7.41 – 38.90	14.9 – 63.00	40 – 45	60.69	NA	48.42	36.20-70.53
CHO (%)	17.92 – 39.95	28.1 – 75.0	NA	10.17	NA	79.84	NA
Calcium (mg/100g)	2.26 – 52.10	76.0 – 78.3	NA	NA	NA	NA	NA
Iron (mg/100g)	28.5 – 54.13	10.6 – 15.1	NA	NA	NA	NA	NA
Zinc (mg/100g)	6.02 – 28.35	7.3–14.0	0.55	0.72 – 0.74	NA	NA	NA
Copper (mg/100g)	1.56	1.0	0.42	0.58 – 0.63	NA	NA	NA
Magnesium (mg/100g)	0.88	530 – 1030	NA	NA	NA	NA	NA
Potassium (mg/100g)	0.23	1182	NA	NA	NA	NA	NA
Sodium (mg/100g)	36.13	2.0	NA	NA	NA	NA	NA
Manganese (mg/100g)	71.12	0.9 – 12.0	1.00	NA	NA	NA	NA

Source: Compiled by author; NA=not available; References used: [1](Sharma *et al.* 2004; Bagheri and Seyedein 2011; Sairam *et al.* 2011; Choi *et al.* 2011; Oluremi *et al.* 2013; Daou and Zhang 2014; Bhosale and Vijayalakshmi 2015; Gul *et al.* 2015; Kaur *et al.* 2017; Begum *et al.* 2018), [2](Shenoy and Prakash 2002, Vitaglione *et al.* 2008; Fardet 2010; Masur *et al.* 2010; Javed *et al.* 2012; Brouns *et al.* 2012; El-Sharnouby *et al.* 2012, Curti *et al.* 2013; Kim *et al.* 2013a; Yan *et al.* 2015; Fleischman *et al.* 2016; Zhao *et al.* 2017; Dhillon and Tanwar 2018; Chalamacharla *et al.* 2018), [3](Dahlberg *et al.* 2004; Kulamarva *et al.* 2009; Sridevi *et al.* 2011), [4] (Sridevi, 2007; Liang *et al.* 2010; Amadou *et al.* 2011; Liu *et al.* 2012; Zhu *et al.* 2018), [5](Bisoi *et al.* 2012; Nazni and Karuna 2016), [6](Bisoi *et al.* 2012; Sarma *et al.* 2017), [7](Bisoi *et al.* 2012; Mustač *et al.* 2020).

Foxtail millet bran dietary fiber have potential to be used as health beneficial ingredients. It was observed that Dietary Fiber (45.79 %) and Insoluble Dietary Fiber (48.36 %) had higher xylose, whereas glucose was higher in SDF (34.56 %). Total phenolic and flavonoid content was highest in DF, followed by IDF and SDF (Dong *et al.* 2019).

Kodo and pearl millet antioxidants protected against both hydroxyl and peroxyl radical-induced DNA scission in dose-dependent pattern. Kodo millet grain extracts showed higher protection against peroxyl radical induced DNA damage compared to corresponding pearl millet extract. Hulls obtained as by products were found to be more effective in the inhibition of DNA strand scission. Hull extract of both kodo and pearl millet exhibited antiproliferative activity superior compared to corresponding dehulled grain extracts, thus suggesting its potential to be used in cancer therapy. Kodo millet hulls extract at 0.5 mg/mL and 0.1 mg/mL inhibited HT-29 cells proliferation as 100 per cent and 99.6 per cent respectively, whereas hull extract of pearl millet at 0.5 mg/mL and 0.1 mg/mL inhibited HT-29 cells proliferation displayed 67.8 per cent and 37.9 per cent respectively. In terms of inhibition against LDL cholesterol hulls displayed a higher inhibition compared to dehulled grains (Chandrasekara and Shahidi, 2011).

The fibre extarcts of kodo, proso, barnyard, finger millet, sorghum and wheat brans were able to bind glucose molecules at different concentrations (5, 10, 25 and 50 milimol/lit) and increase in concentration of glucose led to increase in amount of glucose bound to the fibres. It was also observed that insoluble fibres had effect on delaying glucose diffusion leading to less and delayed absorption of glucose in gastrointestinal tract. This was associated with the viscosity of the fibre. All the fibres obtained from millet (kodo, proso, barnyard, finger millet and sorghum) except wheat bran significantly retarded starch digestibility and decreased alpha-amylase activity, indicateing insoluble fibres from millet may have hypoglycaemic properties (Bisoi *et al.* 2012).

Normal diet (Test Diet No. 58124) high fat diet (HFD) (Test Diets No. 58125) incorporated with (20 % dry weight) kodo millet whole grain (KM-WG) or kodo millet bran (KM-BR) was fed to mice. Incorporation of KM-WG and KM-BR averted further increase in lipid and serum cholesterol and improved the glucose tolerance. No improvement was seen in oxidative stress parameters in liver, visceral white adipose tissue, subcutaneous white adipose tissue, muscle or pancreas. The beneficial microorganisms viz., *Lactobacillus sp.*, *Bifidobacteria*, *Roseburia* spp. and *A. muciniphila* increased with KM-WG and BR supplementation (Sarma *et al.* 2017).

Wu *et al.* (2011) assessed biological properties of procyanidin-rich extract (PARE) from sorghum bran in mice. PARE contained 86.9 per cent total phenolic and 54.68 per cent total procyanidins content. PARE at 150 mg/kg effectively scavenged free radicals and significantly ($p < 0.05$) suppressed production and rise in MDA levels in liver homogenate and serum. SOD (superoxide dismutase) a main component of antioxidant defence system was restored significantly in liver and serum at the dose of 150 mg/kg of PARE. PARE also significantly increased GPx activity. It was also able to prevent the D-galactose-induced oxidative stress. Inhibition of tumor weight was attained with administration of PARE at doses of 100, 200 and 400 mg/kg with inhibition rates of 8.14 per cent, 32.21 per cent and 52.30 per cent ($p < 0.01$) respectively. Thus, PARE had antioxidant and antitumor activities.

Anthocyanin apigenindin and luteolindin identified from acidified methanol extract of sorghum millet bran inhibited the growth of HT 29 colon cancer / HEP G2 in concentration dose dependent manner with maximum cytotoxity at 500 µg/ml after 72 h treatment. This clearly indicated that red sorghum bran extract can show modulatory effect on cell proliferation, cytotoxicity and oxidative reactions in cellular systems due to the presence of anthocyanin (Kumari *et al.* 2013).

In sorghum bran (SB) nine 3-deoxyanthocyanins (DA) were identified. Out of various Glucoarabinoxylans (GAX) extracts from white, red and high tannin SB, high tannin SB depicted higher antioxidant capacity due to its tannin content. The gelling capacity of these brans was weak or no gel was formed due to presence of DA (Ayala-Soto *et al.* 2015).

Finger millet whole (FM-WG) and bran (FM-BR) was supplemented to high-fat diet-fed (HFD) Swiss albino mice for 12 weeks. At end of 12 weeks FM-BR group of mice showed significant decrease in body weight compared to other groups. Glucose clearance was impaired in HFD group but it was enhanced in FM-BR group. No difference in cholesterol, and serum lipids were observed in any group. Lipid peroxidation increased in HFD group, but it was prevented in FD-BR-fed and HFD-WG-fed mice. Increase in catalase levels in vWAT and liver was seen in HFD-BR-fed mice. FM-BR at 10% (w/w) had more beneficial effects than that of FM-WG. Thus, it was concluded that finger millet bran supplementation can prevent body weight gain, improve lipid profile and anti-inflammatory status, reduce oxidative stress, modulate the expression of several obesity-related genes, boost the proliferation of beneficial gut bacteria (*Lactobacillus,* *Bifidobacteria* and *Roseburia*) and inhibit *Enterobacter* in caecal contents (Murtaza *et al.* 2014).

Arabinoxylan (AX) extracted from finger millet bran had ferulic content. Nitric oxide (NO) production which plays important role in the killing of intracellular microbial pathogens and tumoricidal activity, increased in group of mice treated with AX from finger millet bran. Thus, finger millet bran can be exploited as immunomodulator due to its mitogenic activity, activation of macrophages and phagocytosis (Prashanth *et al.* 2015).

Whole grain showed inverse relation with weight gain and addition of bran fortified diet (wheat bran, corn bran, oat bran, rice bran, and wheat germ) further reduced risk of weight gain. The reduction in weight gain was dose dependent, with increase in consumption of whole grains by 40 g per day the weight gain reduced by 0.49 kg. The bran rich diet further reduced the risk of weight gain by 0.39 kg for increased intake of every 20 g per day (Koh-Banerjee *et al.* 2004).

In a randomized controlled 5-week crossover intervention with 25 subjects high fibre (HF), low fibre (LF) diet with total dietary fibre 48.0 g and 30.2 g per day was given. High fibre diet included oat bran, rye bran, and sugar beet fibre into test food products and equivalent foods without fibre were given for LF diet. The results indicated significant reduction in C-reactive protein (CRP) and fibrinogen levels during HF diet and not in low fibre diet. Thus, it was concluded that high fibre diet can help to prevent the risk of cardiovascular disease (Johansson-Persson *et al.* 2014).

Double blind clinical trial conducted on 60 diabetic patients, with one group receiving 10 g soluble rice bran twice a day for 30 days and another group as placebo showed that fasting serum glucose level and 2 hours after food were significantly reduced for intervention group compared placebo. Triglyceride reduced significantly and HDL increased in intervention group compared to placebo group, LDL and cholesterol also decreased but the reduction was not significant (Takakori *et al.* 2004).

Significant reduction in blood glucose levels in both groups of mice fed with high fat diet supplemented with rice bran or phytic acid when compared to mice fed only with high fat diet or the control group, concluding that rice bran and phytic acid can reduce the risk of hyperglycemia induced by high fat diet. It was reported that rice bran and phytic aicd might have enhanced glucose metabolism due to the activation of GK, and inhibition of G6pase and PEPCK enzymes in the liver (Kim *et al.* 2010).

A study for 12 weeks on supplementation of rice bran (70 g) and rice husk (25 g) to two groups (n=35) and control following energy restricted diet providing total 28 g of fibre per day indicated that energy restricted diet coupled with rice bran or rice husk had positive effect on inflammatory markers among overweight and obese adults. After supplementation there was reduction in weight, waist circumference, BMI and energy intake compared to the baseline data. Inflammatory factors such as serum levels of IL-6 and hs-CRP decreased significantly in rice bran and rice husk group (Edrisi *et al.* 2017).

Another study on 48 female participants divided in in 4 groups viz., control (C), oat bran supplementation alone (Ob), Exercise alone (Ex) and combined oat bran and exercise (ObEx) groups showed that total cholesterol, triglyceride and low density lipoprotein cholesterol reduced significantly in ObEx. Oat bran supplemented daily to both groups Ob and ObEx was 18 g/day (Ooi and Ridzuan 2016).

In another study commercially available oat bran (OatWell®22) containing 22 per cent of high molecular weight oat β -glucan was mixed in water given to 10 subjects and glycemic response after its consumption was checked. It was observed that consumption of 27.3 g commercially available oat bran significantly reduced iAUC when compared to white bread used as reference food (Steinert *et al.* 2016).

Wolever *et al.* (2018) also concluded that β-glucan from oat bran added to instant oat meal reduced post prandial glucose levels after supplementation in dose dependent manner. β-glucan from oat bran of 1.6 g was required for reducing the iAUC by 20 per cent.

Supplementation study on in women with gestation diabetes mellitus revealed that, subjects who received low glycemic diet coupled with wheat bran (15 g) reduced the number of subjects needing insulin treatment (Afaghi *et al.* 2013).

Immature wheat bran extracts were identified to be effective against cancer cells. The IWB extracts increased the gene expression of p53 and PTEN (tumour suppressor genes) in HT-29 cells. IWB extracts also demonstrated the highest inhibition of Caco-2, HT-29, and HeLa cells. It inhibited cell growth and induced apoptosis showing anti-cancer properties (Kim *et al.* 2016).

2.5 Stabilisation of millet and cereal bran

Cereal brans as functional foods are emerging due to their health promoting components such as fibre, minerals, antioxidants etc. and are used for value addition of various products. The main hindrance on using the brans is their short shelf life as it deteriorates faster (Sharma *et al.* 2014). Thus, stabilisation of bran is important to inactivate the lipase activity and increase the shelf life by decreasing rancidity. Several protocols for stabilisation are followed by various authors viz., dry heat, moist heat, microwave, extrusion and also the use of chemicals. Bran is also processed by various methods of bioprocessing such as enzymatic and fermentation treatments to improve its nutritional properties (Coda *et al.* 2015). Summary of bran stabilization is presented in Table 2.2.

Rice bran stabilised with 3 min at 100 per cent power in a preheated microwave oven (850 Wand 2450 MHz) was stored for 16 weeks at 4 – 5 °C and 25 °C and there was no significant changes in protein, fat, linoleic, and linolenic acid compared to raw bran during storage (Ramezanzadeh *et al.* 2000).

Stabilising of rice bran was carried out using hot air oven (HA) at 120 °C for 30 minutes and extrusion stabilisation (ES) with following conditions: water flow rate: 0.000038m3/s, feed rate: 27 kg/hr, steam supply: 275.80 kPa, die opening: 0.0078 m, temperature: 135–140 °C. Stabilised bran samples were stored at ambient room temperature and FFA was recorded at 0, 15, 30, 45 and 60 days. FFA increased during 60 days storage for all brans, but the increase was less in stabilised bran compared to bran not stabilised. FFA noted at 0 day was 4.05, 3.66 and 3.85 for raw, HA and ES bran which increased to 64.60, 9.15 and 4.10 at 60th day respectively showing ES was best amongst the treatments for bran stabilization (Sharma *et al.* 2004).

Oxidative stability of rice bran increased when it was heated in hot air oven for 30 minutes at 120°C. It was also found that headspace oxygen content decreased significantly in untreated rice bran as compared to heat treated rice bran during storge period of 12 days. In heat treated rice bran the decrease in oxygen was not significant when compared between day 0 to 12th day of storage. Consumption of head space oxygen gives indication of lipid oxidation, more the oxygen consumed more is the lipid oxidation rate (Kim *et al.* 2013b).

Effects of various treatments viz., autoclaving and drying overnight at 80 °C, heating in Hot Air Oven (HAO) at 130 °C for 20 min and 180 °C for 5 min, Microwave (MW) heating at 900 Watt for 1 min, 1.5 min, and 2.5 min and soaking with 80 per cent Et-OH for 6 hours, filtrating followed by drying at 80 °C on rice bran stability showed that MW heating (900W) for 2.5 min was best among the treatments providing higher stability in respect of the antioxidant activity, ɤ-oryzanol, crude oil and total protein content and gave minimum rancidity when preserved for 180 days (Bagchi *et al.* 2014).

Similarly, changes in FFA for microwave stabilised and probiotic treated rice bran were compared with control rice bran during the storage period of 4 weeks. It was observed that FFA was least in microwave stabilised rice bran (7.50 %), followed by probiotic treated rice bran (7.95 %) and highest FFA was noted in control (unstabilised) rice bran (24.70 %). This depicted that microwave heating was most suitable for rice bran stabilisation (Bhosale and Vijayalakshmi, 2015).

Patil *et al.* (2016) also stabilized raw **rice bran using** microwave power (2, 4, 6 W/g) at different exposure time (1, 3, 5 min) and compared it with parboiled, unstabilised rice bran. It was found that microwave power level of 4 W/g for 5 min was a most effective treatment in stabilising rice bran considering all the parameters viz., FFA, oil yield, oil quality, moisture and protein. It was noted that during the storage period of 90 days microwave stabilised bran was stable with minimum (%) FFA, peroxide value (PV) and Thiobarbituric acid (TBA) number followed by parboiled rice bran whereas control was not stable with highest values FFA, PV and TBA.

Similar reports were quoted in a study conducted **on rice bran using** microwave assisted stabilisation techniques and investigated its effect on the quality of extracted rice bran oil (RBO) from different varieties viz., lowland, upland and Bario. The stabilisation was carried out in a 1000 W output microwave oven by placing the sample for 3 minutes at 40 per cent power. The process was repeated thrice to ensure complete stabilisation and stabilised rice bran was stored in 4 °C. During 40 days storage, there was an increase in moisture content, decrease in oil yielding leading to increase in free fatty acid (FFA) contents in all the varieties, indicating continuous hydrolysis and oxidation of the oil in rice bran to free fatty acids along the storage time even at low temperature (4 °C). Moisture content, associated with an increase in FFA, increased more in unstabilised than stabilised bran. Thus, it was concluded that microwave stabilisation significantly improved the properties of rice bran by reducing the moisture content during storage (Daud *et al.* 2017).

Rice bran stabilised using microwave heating (MH-1, 2: 100 % power, 1–2 min) and dry heating (DH-10, 20: 120 °C, 10–20 min) was compared with control (unstabilised rice bran) during storage at room temperature in polyethylene bags. It was observed that FFA increased during storage much higher in unstabilised bran than stabilised bran indicating that stabilisation was effective in inhibiting enzymatic degradation. The FFA contents of the control, DH-10, DH-20, MH-1 and MH-2 were 39.51, 15.85, 14.47, 11.64 and 10.67 mg KOH/g after 35 days of storage respectively (Lv *et al.* 2018).

After microwave and hot air oven heating, another alternative effective stabilisation treatment was ohmic heating reported by researchers to stabilised rice bran. During six weeks storage study Lakkakula *et al.* (2004) observed that FFA concentration increased slowly in rice bran samples subjected to ohmic heating than control. Ohmic heating at 10.5 per cent 1 Hz; 10.5 per cent 60 Hz, 21 per cent 1 Hz; 21 per cent 60 Hz had FFA 3.81±0.01, 3.83±0.14, 3.07±0.12 and 3.25±0.01 at 0 day which increased to 11.27±0.35, 12.2±0.03, 5.54±0.01 and 5.47±0.01 per cent respectively during 6th week. Microwave stabilisation at 21 % for 3 minutes was better compared to control where the FFA increased from 2.8 ±0.03 to 3.89±0.26 during six weeks. Whereas the control sample had 3.96±0.13 FFA at 0th day which increased to 18.03±1.22. It was concluded that both ohmic heating and microwave stabilisation was suitable for rice.

Similar outcomes were noted when raw untreated rice bran was compared with Ohmically stabilised rice bran after 75 days of storage. The increase in FFA (%) for ohmically treated bran was observed to be 4.77 per cent after 75 days of storage whereas it was 41.84 per cent in case of raw bran (Dhingra *et al.* 2012).

Loypimai *et al*. (2015) through their work stabilised rice bran using ohmic heating and steaming which was further extracted to obtain rice bran oil using 3 treatments viz., immersion stirring method, Soxhlet extraction method, and the enzymatic extraction method. Out of all the extraction treatments, Soxhlet extraction of ohmically stabilised rice bran yielded the highest amount of oil (17.11 %) compared to bran stabilised using steaming treatment (15.10 %), least oil recovery was from immersion stirring extraction of bran stabilised with steaming. Stabilisation significantly affected the FFA, content of extracted oil, whereas no significant difference was seen in peroxide value (PV), thiobarbituric acid value (TBA) of brans stabilised. It was observed that both steamed and ohmically treated rice bran further extracted enzymatically had least FFA, PV and TBA content.

Chemical treatments were also used for stabilisation of bran via, inactivation of lipase. Acid stabilisation of rice bran with HCl (30 per cent: v/v) at the rate of 44 ml per kilogram, and heat stabilisation at 120 °C for 10 to15 seconds was reported to have better effects (Younas *et al.* (2011). Inactivation of lipase and stabilisation of rice bran improving its shelf was achieved by the application of chemicals Hydrochloric Acid (HCl) at 30 ml/Kg, Phosphoric Acid at 1.5 % W/W, Acetic Acid at 7 % W/W and Sodium Metabisulphite at 2 % W/W. It was observed that the use of HCl effectively inactivated the lipase activity during storage period of 60 days at room temperature (Akhter *et al.* 2015).

Along with rice bran attempts were made to utilise all these treatments for stabilising other cereal and millet brans by different researchers.

Stabilisation of wheat bran revealed that 50 per cent lipase activity was reduced when wheat bran subjected to steam heat-treated bran (SHTB) and dry heat treatment (DHTB) at 100 °C for 3-4 hours and the bran remained stable for 3 months without developing rancidity or bitter flavour. Increase in FFA during 90 days storage was significantly higher for untreated wheat bran (10.5% to 35.61%) compared to SHTB (6.9% to 7.99%) and DHTB (6.0% to 7.12%). Peroxide value also showed a significant increase in untreated wheat bran in comparison to treated bran samples SHTB and DHTB. Also, the lipase activity increased during storage from 780 mmol to 1150 mmol in untreated bran whereas in SHTB it increased from initial 320 mmol to 551 mmol, and for DHTB, the initial value of 390 mmol had increased to 588 mmol (Sudha *et al.* 2011).

Another study on wheat, rice, barley and oat brans stabilisation by using various treatments viz., wet heating at 115 °C for 10 and 15 min., dry heating at 110 °C for 25 min., microwave heating at 2450 MHz for 2.5 min., extrusion cooking at 140°C with 20 per cent moisture content and chemical treatment (1% acetic acid solution at 22%) extended the shelf life of brans, with extrusion technology giving better outcomes. The mean free fatty acids (FFA) in the cereal brans subjected to extrusion cooking, microwave heating, chemical treatment, wet and dry heating was 3.14, 3.76, 4.08 and 4.44 and 5.94 per cent, respectively (Sharma *et al.* 2014).

Devittori *et al.* (2000) reported that proso millet bran containing 7–8 per cent moisture and 7 per cent oil, which was used either directly or after drying for 3 h at 105 °C (residual moisture 1–2%). The oil from proso millet bran was either extracted with supercritical carbon dioxide (SC-CO_2) or Soxhlet to yield crude oil, both the extracts were compared for free fatty acid profiles. Similar values were observed for FFA (~12%) and peroxide value (3–6 meq O2/kg) in Soxhlet and SC-CO_2 oil.

Table 2.2. Summary for stabilisation of cereal and millet brans

S. No	Bran	Stabilisation treatments	References
1.	Rice bran	Autoclaving, Hot Air Oven heating, Microwave heating, Chemical treatment (acetic acid, Et-OH, HCl) Extrusion cooking, Ohmic heating and Steaming	Ramezanzadeh *et al.* (2000); Sharma *et al.* (2004); Lakkakula *et al.* (2004); Dhingra *et al.* (2012); Sharma *et al.* (2014); Bagchi *et al.* (2014); Akhter *et al.* (2015); Bhosale and Vijayalakshmi (2015); Loypimai, *et al*. (2015); Patil *et al.* (2016); Egbedike *et al.* (2016); Daud *et al.* (2017); Rafe *et al.* (2017); Lv *et al.* (2018)
2.	Wheat bran	Steam treatment, Dry heating treatment, Microwave heating, Extrusion cooking, chemical treatment (acetic acid)	Sudha *et al.* (2011); Sharma *et al.* (2014); Ertas (2015); Lauková *et al*. (2019)
3.	Barley bran	Dry heating treatment (hot air oven), Microwave heating, Extrusion cooking, Chemical treatment (acetic acid)	Sharma *et al.* (2014)
4.	Oat bran	Dry heating treatment, Microwave heating, Extrusion cooking, Chemical treatment (acetic acid)	Sharma *et al.* (2014)
5.	Barnyard millet bran	Chemical treatment	Nazni and Karuna (2016)
6.	Proso millet bran	Hot Air Oven heating	Devittori *et al.* (2000)

(Source: Compiled by author using previous studies)

2.6 Formulation, sensory evaluation and physicochemical properties of designer products incorporating millet and cereal bran

Consumption of fibre and fibre rich food is still below the recommended allowances. Thus, value addition of foods with fibre derived from various sources viz., cereals, fruits, vegetables etc., can be alternative solution to fill gap between prevailing consumption levels and recommended intake of fibre (Heinio *et al.* 2016). The current situation also depicts that bakery items such as biscuits, cakes, muffins, buns, breads etc. have become popular in all age groups across the globe (Lebesi and Tzia, 2011) but all these products lack fibre as they are prepared using refined flours, making it an ideal choice for incorporating fibre. But the challenge is developing fibre rich or high fibre bakery product acceptable and palatable for the consumers, as addition of fibre alters the sensory parameters resulting in reduced acceptance (Li and Komarek, 2017). On one hand bakery items are becoming more popular amongst the consumers, at the same time there is a growing awareness about nutrition, demanding healthy and nutritious foods. Thus, it paves way to develop innovative fibre enriched bakery items incorporating fibre from various sources. Various researchers are attempting to develop such innovative products by replacing refined wheat flour and whole wheat flour with cereal brans to make available products tasty palatable and healthy.

2.6.1 Cookies and biscuits

Defatted rice bran (DRB) incorporation upto 10-20 per cent replacemenet in cookies were acceptable, beyond that, the colour of the cookies became darker and flavour affected thus lowerd sensory acceptability (Sharif *et al.* 2009).

Biscuits blended with wheat, soybean and rice bran flour at various ratios viz., 100:0:0, 70:20:10, 50: 30: 20, 30: 40:30 and 10:50:40 respectively when evaluated on 9-point hedonic scale depcited that biscuits with 100 per cent wheat were best acceptable, followed by biscuits with 20 per cent soybean and 10 per cent rice bran flour (Bunde *et al.* 2010).

Rice bran cookies up to 10 per cent rice bran were acceptable. The thickness of cookies increased with increased addition of rice bran, whereas the width and spread ratio decreased. The nutritional profile also improved after addition of rice bran and as the per cent incorporation increased the nutritional profile improved (Younas *et al.* 2011).

Precooking of wheat flour with 0 – 30 per cent wheat bran using extrusion technology resulted in increase in soluble dietary fibre and decreased total dietary fibre. Cookies and tortillas prepared using these uncooked wheat flour and precooked wheat flour with bran incorporation at 0-20 per cent resulted in reduced consumer acceptability as bran (%) increased. Cookies scored between 6 – 7 for both precooked and uncooked flour with 20 per cent bran, whereas the tortillas scored between 5 – 6 for both precooked and uncooked flour with 20 per cent bran. In both the products precooked flour with 20 per cent bran received the least scores. Increase in bran levels resulted in increased darkness of the products (Gajula *et al.* 2008).

Addition of wheat bran (WB) and date powder (DP), increased water absorption of biscuit dough but decrease dough stability. Breaking strength and colour difference increased with increased addition of WB and DP, but the diameter, spread ratio and whiteness decreased. Sensory evaluation revealed that all the products were acceptable but the scores decreased as the per cent of wheat bran and date powder increased. Twenty per cent of wheat bran and date powder incorporation was most acceptable after control (El-Sharnouby *et al.* 2012).

Cookies were prepared with hot air oven (150 °C for 20 min), domestic microwave oven (800W for 3 min) and autoclave (121°C for 90 min) stabilised wheat bran (WB) at various levels viz., 10, 20, 30 %. Hot air oven stabilised WB at 10 % gave best sensory scores. Color did not change significantly between three stabilisation treatments but incorporation of bran at 30 % in cookies decreased lightness and increased redness (Ertas, 2015).

Various combination of refined flour: rice bran and refined flour: wheat bran cookies (50:50, 60:40, 70:30, 80:20, 90:10). It was observed that cookies with 30 per cent bran replacement scored best amongst all the treatments (Suma and Nandini, 2015).

Another study revealed that cookies developed with 5 per cent stabilised wheat bran was acceptable compared to 10 -15 per cent incorporation of wheat bran. Addition of wheat bran increased the water absorption properties of flour (Lauková *et al*. 2019).

Cookies developed with blending barely malted bran (BMB) at 5 to 50 per cent in wheat flour resulted in increased the weight, diameter and thickness of cookies and reduced the baking strength compared to control (no bran) cookies. Addition of BMB significantly reduced sensory scores. Cookies with 5 per cent incorporation had highest sensory scores after control cookies (Ikuomola *et al.* 2017).

2.6.2 Pasta and extruded products

Pasta was prepared with addition of commercially available dietary fibre viz., inulin, guar gum and pea fibre by replacing wheat flour at 7.5, 10, 12.5, and 15 per cent. Addition of guar gum increased the swelling properties of pasta, cooking losses increased with addition of inulin and pea fibre compared to control, but decreased in pasta with guar gum. Firmness of pasta significantly reduced with pea fibre at 7.5 and 15 per cent and guar gum at 10 per cent but rest all the variations had no significant change (Tudoricaÿ *et al*. 2002).

Addition of dry and steam heat treated wheat bran at 40 per cent and 50 per cent in semolina developed high fibre pasta without affecting cooking quality and sensory scores. Firmness of pasta was measured using texture analyser and it was noted that increase in bran per cent decreased the firmness this was associated with diluting effect of bran on gluten cohesiveness (Sudha *et al.* 2011).

Deoiled rice bran (DRB) used for preparation of extruded snacks resulted in increase of water holding capacity of DRB in extruded product (EP). The oil absorption capacity also increased to 315.2 in the EP from 278.9 in DRB. The extruded snack was prepared using 17.73 per cent deoiled rice bran, 10 per cent corn flour and 72.27 per cent rice flour (Sharma *et al.* 2018).

Polysaccharide extracted from oat bran when extruded showed better solubility, solvent retention capacity and water binding capacity. Extensibility and gumminess of the extruded oat bran polysaccharide dough improved. It was concluded that extruded oat bran polysaccharide can be used as functional ingredients (Wu *et al*. 2018).

2.6.3 Breads and buns

Supplementation of 15 per cent sorghum bran in breads resulted in excellent phenolic and antioxidant content without hampering the physical properties like specific volume, loaf appearance. Colour of the bran added brownish dark colour to loaves (Dahlberg *et al.* 2004). Similarly, bread containing 12 per cent brown sorghum bran 5 per cent flaxseed and 2 per cent soya flour showed better dietary fibre, antioxidants and better palatability adding dark brown colour to the loaves (Hines, 2007).

Sensory comparision of native, fine, coarse, xylanase-treated, and cellulase treated sorghum and millet (MB-87) brans incorporated at 20 per cent in breads showed that enzymatically treated bran-supplemented breads had higher scores for overall acceptability compared to other brans, suggesting that modification of bran positively alters bran morphology making it acceptable for consumers (Ahmad *et al.* 2018).

High fibre breads by addition of psyllium husk and oat fibre at 0, 1, 2, 3, 4 and 5 per cent resulted in increased baking absorption and decreased loaf volume by 10 per cent with the fibre enriched buns. Oat fibre incorporation at 5 per cent was best accepted by panellist after control and psyllium husk at 3 per cent was best accepted (Kamaljit *et al.* 2011).

Breads enriched with rice bran at 0, 5, 10, 15 and 20 per cent revealed that incorporation up to 10 per cent was acceptable. With increase in per cent of bran, sensory acceptability decreased. Gravimetric characteristics showed that as per cent bran increased there was increase in water absorption, weight and decrease in volume. Farinograph showed decrease in dough mixing stability with increased bran incorporation (Bagheri and Seyedein, 2011).

Defatted rice bran enriched breads (5, 10 and 15 %) showed that breads with 5-10 per cent incorporation were found more acceptable compared to 15 per cent. Breads with 15 per cent bran incorporation was found acceptable by addition of bread improvers (Sairam *et al.* 2011). Similar results were depicted by Curti *et al.* (2013) where addition of rice bran (13-23.5 %) reduced the loaf volume and increased hardness of the bread compared to breads with no bran.

Egbedike *et al.* (2016) studied the effect of replacing rice bran in water yam flour at 10, 20, 30 and 40 per cent. It was noted that as percent rice bran increased water absorption, oil absorption and emulsion capacity increased but the foaming capacity decreased.

Sangak a sourdough flat bread popular in Iran was prepared by incorporating hydrothermaled wheat bran. Hydrothermal treatment of bran decreased the phytate content by 57 per cent in flour and 55 per cent in dough. Addition of bran slightly increased water absorption capacity of the flour, had higher valorimetric value, longer development time, more stability and lower degree of softening ($p<0.05$). Thus, hydrothermal bran addition improved the dough rheological properties (Mosharraf *et al.* 2009).

Wheat bran with nutritional composition of 8.9 % moisture, 14 % protein, 5.9 % ash, 10.1 % fibre and 3 % fat was incorporated in bread. The incorporation of bran (10

%) with gluten, Glycerol Mono Stearate and Sodium Stearyl improved the baking quality of bread. But bran alone decreased the loaf volume (Masur *et al.* 2010). Another report on addition of wheat bran in bread making also stated that hardness of breads increased and volume of loaf decreased in proportion of bran incorporation (Lehtinen, 2012)

Addition of bran negatively affected the sensory quality of breads, but when the bran particle size was reduced and organogel was added, it positively influenced sensory parameters increasing the sensory scores making 15 per cent wheat bran incorporated breads acceptable (Saccotelli *et al.* 2017).

Kaur *et al.* (2017) prepared buns, pizza base and flatbread by incorporating hot air oven stabilised (110°C for 25mins) cereals brans viz., wheat bran (WB), oat bran (OB) and rice bran (RB) at various levels (5, 10, 15 %). The loaf volume decreased with increased bran per cent and the loaf weight increased with increase in bran per cent. Increase in redness and decrease in yellowness was observed with increased bran supplementation making the product darker and redder. All products prepared with bran had harder texture compared to refined wheat flour buns. Sensory evaluation revealed that all products made with 5-10 per cent WB, OB were highly acceptable whereas RB was acceptable at 5 per cent.

Breads made with 50 per cent bioprocessed wheat bran (WB) incorporation were found to be unacceptable by trained sensory panel, whereas the breads with 30 per cent WB could get better scores compared to 50 per cent incorporation, but breads with 10 per cent WB having 400 and 750 μm particle size scored very well in the sensory parameters (Bartalné-Berceli *et al.* 2018).

Fermentation of wheat bran reduced the negative effect of bran addition on farinographic and extensiogarphic properties. Fermentation of bran also had positive influence on the bread volume (Li *et al.* 2018).

Wheat bran dietary fibre (WBDF) also showed similar adverse effects on the dough. Pasting properties showed reduction in peak viscosity, trough viscosity, final viscosity as percent WBDF increased. Farinographic properties of flour showed that as WBDF increased, water absorption and formation time increased and it also increased weakening degree (Liu *et al.* 2019).

Breads developed by substituting wheat flour with rice, wheat and barley bran at 10, 20, 30 per cent showed decrease in loaf volume and specific loaf volume. The L, a, and b values decreased as the concentration of bran increased. Lower L value than control resulted in darker crust for the bread with bran. Bran incorporation at 30 per cent resulted in harder bread. With increase in per cent bran incorporation all the sensory scores decreased, bran incorporation up to 10 per cent was acceptable at sensory level (Hussein and Ibrahim 2019).

Cereal fried dough (*magwinya*) supplemented with wheat bran (WB) at 5, 10, 15 and 20 per cent resulted in darker crust and crumb as the per cent of bran increased. Hardness increased as the per cent of bran increased. *Magwinya* with 5 per cent incorporation for WB was highly acceptable by consumers compared to other variations (Ndlala *et al.* 2019).

Oat and wheat bran enriched chicken patties was acceptable up to 10 per cent (oat) and 15 per cent (wheat) bran respectively were acceptable. Increase in water holding capacity and emulsion stability of chicken meat emulsions was reported after addition of wheat and oat bran (Talukder and Sharma, 2010).

2.6.3.1 *Chapati*

Chapati are Indian flat breads consumed daily during meal times especially in north India. *Chaptis* are commonly prepared with whole wheat flour (*atta*), obtained from local mills or '*Chakki'* (Dar *et al*. 2014). But practice in many household is to sieve the flour before using it for chapati preparation thus removing bran (Rao and Manohar, 2003). Authors have reported preparation of chapati by addition of cereal and millet bran.

Composite brown flour developed by enriching wheat bran (0, 5, 10, 15 and 20 %) and preparing *chapatis* using this composite flour resulted in acceptable *chapatis* habing bran up to 10 per cent and it was on par with the whole what flour (Butt *et al.* 2004).

Chapati prepared by addition of rice bran, wheat bran and oat bran were acceptable at 5 – 10 per cent (Dar *et al*. 2014).

Chapatis prepared with whole Wheat Flour (80 g) incorporated with Foxtail Millet Bran (FMB) at 19.98 gm scored 7 for overall acceptability textural and sensory properties (Nazni and Gomathi, 2015).

2.6.4 Muffins and Cupcakes

Cupcakes developed by incorporation of 10, 20, 30 per cent of cereal brans viz., rice, wheat and oat affected the sensorial parameters decreasing its volume, porosity, crumb moisture and batter viscosity. It was noted that sensory acceptability declined with increase in addition of bran. Wheat, rice and oat bran contributed to husky taste. Ten per cent of cereal bran incorporation was found to be best acceptable (Lebesi and Tzia, 2011).

Fibre enriched muffins were developed by replacing wheat bran at 8, 16, 24 per cent, carrot powder at 10, 20, 30 per cent and ground Bengal gram at 12, 24 and 36 per cent in wheat flour. Wheat bran incorporated muffins were more acceptable and closest to control followed by carrot powder incorporated muffins. Muffins with 8, 16 and 24 per cent wheat bran scored 6.47, 6.33 and 6.13 respectively whereas control scored 6.83 (Romjaun and Prakash, 2013).

Nazni and Karuna (2016) developed rusks and muffins by addition of barnyard millet bran (BMB) from 0, 5, 10, 15, 20, 25 and 30 per cent in wheat flour. Hardness of rusk and muffin increased with addition of BMB. Rusk at 30 per cent and muffin at 15 per cent were acceptable.

Muffins developed with functional ingredients viz., 25 per cent defatted soya flour, 75 per cent whole wheat flour, Ginger powder: Wheat Bran: Tomato pulp in 1:4:8 ratio and black pepper: turmeric in 1:2 ratio had highest overall acceptability (8.0 ± 0.47) followed by control (7.5±0.58). Appearance colour, texture was more preferred in functional muffin than control muffin (Dhillon and Tanwar, 2018).

2.6.5 Products prepared using other sources of fibre

There is an emerging trend of incorporating other fibre sources in bakery items other than millet or cereal bran for improving their nutritional profile. Some researches are reported under following section.

Biscuits incorporated with mango peel powder (MPP) at 2.5, 5.0, 7.5 and 10 per cent replacing wheat flour resulted in increased, water absorption of dough, dough development time also increased. But strength of dough reduced with increased addition of MPP. Diameter of the biscuits significantly reduced with 20 per cent incorporation, but there was no significant difference up to 10 per cent. L, *a*, *b* values depicted that colour was brighter for control but brightness decreased with addition of MPP. The biscuits with

10 per cent were sensorially acceptable but acceptability decreased for 15 and 20 per cent due to bitter taste given by MPP (Ajila *et al.* 2008).

Carrot pomace (CP) a by-product obtained from carrot processing was powdered and incorporation in wheat rolls (0 %, 1 %, 3 %, 5 % and 10 %). Water absorption, dough development time and dough stability increased with addition of CP powder. Loaf volume, cambering and over all acceptability decreased significantly with increase of CP powder. Incorporation up to 3 per cent was acceptable by sensory panel (Kohajdova *et al.* 2012).

Apple skin powder (ASP) incorporated in muffins at 0, 4, 8, 16, 24 and 32 per cent resulted in increased firmness and decreased volume of muffin. It was noted that 32 per cent incorporation deteriorated the sensory and baking charateristics. ASP up to 24 per cent gave acceptable result in sensory and baking characteristics (Rupasinghe *et al.* 2009).

Fruit by-products viz., Raspberry Pomace Powder (RPP) and Cranberry Pomace Powder (CPP) were used at 10, 20per cent to develop muffins. It was revealed that hardness of muffins increased with addition of RPP and CPP. The longer baking time (240 °C for 15 min) favoured better release of ellagic acid, but had negative impact on the flavanols and anthocyanins (Mildner-Szkudlarz *et al.* 2016).

Cabbage outer leaf powder, a kimchi by-product (KBP) was used to develop dietary fibre enriched muffins at 1-4 per cent . It was revealed that addition of KBP resulted in decreased acceptance due to increased hardness. Sensory scores for overall acceptability also decreased with increasing ratio of KBP, with no significant difference observed at 2per cent incorporation (Heo *et al.* 2019).

Buns prepared by 4, 6 and 8 per cent substitution of soy fibre (Okara) influenced the colour, the darkness, redness and yellowness increased with addition of bran compared to control. There was a significant decrease in scores for uniformity, crust characteristic, crumb- cell structure, colour, texture and aroma for soy fibre enriched buns (Bhavya and Prakash, 2018).

Another study reported use of defatted apple seed powder (DASC) in value addition of bread. It was observed that 5 per cent addition of DASC did not affect the colour and sensory parameters compared to control but incorporation 20 per cent DASC resulted in darker bread and significantly reduced sensory scores. Twenty per cent DASC addition resulted in imparting slight bitter taste to breads (Purić *et al.* 2020).

2.7 Nutritional composition, glycaemic index, glycaemic load and therapeutic potential of designer products incorporated with millet bran, cereal bran and other sources of fibre

Cookies prepared with defatted rice bran (DRB) incorporation resulted in cookies with increased dietary fibre content compared to control cookies. Mineral content also increased for potassium, calcium and magnesium with increased level of DRB (Sharif *et al.* 2009).

Biscuits developed by replacing wheat flour with 20 per cent soybean and 10 per cent rice bran flour were found to have higher protein (16.28 %), fibre (1.90 %) and fat (12.13 %) as compared to whole wheat biscuits (Bunde *et al.* 2010).

Rice bran protein concentrates (RBPC) added to biscuits at 5, 10 and 15 per cent upgraded the nutritional value of normal biscuits. Analysis of RBPC and defatted rice bran showed that higher ash, fat, crude fibre and carbohydrate content was present in defatted rice bran and higher protein was in RBCP. Sensory scores revealed that 5 % biscuits were closer to control biscuits and with increasing per cent of RBCP the sensory scores decreased (Yadav *et al.* 2011).

Addition of rice bran in water yam flour at 10, 20, 30 and 40 per cent increased the protein, ash, oxalate, saponin and alkaloid content of the flour (Egbedike *et al.* 2016).

Biscuits prepared by incorporation of wheat bran and date powder showed increased amount of protein, crude fibre, ash and minerals compared to control biscuits (El-Sharnouby *et al.* 2012).

Wheat bran incroported cookies (10 to 30% incorporation) revealed that protein and ash content increased in cookies enriched with stabilised wheat bran. 30 % bran incorporation had highest content of minerals (Ca, K, Mg, Mn, Na, Cu, P, Fe and Zn) as compared to other levels of incorporation and control cookies (Ertas, 2015).

Cookies prepared by incorporating barley malted bran (BMB) up to 50 per cent increased the nutritional content compared to control cookies. Ash content increased from 1.41 to 1.88 per cent, protein from 11.21 to 15.64 per cent, crude fibre from 1.32 to 6.38 per cent and decreased carbohydrate content from 52.79 to 40.05 per cent (Ikuomola *et al.* 2017).

Biscuits developed with Mango peel powder (5.0, 7.5, 10.0, 15.0 and 20.0%) increased total dietary fibre content from 6.5 in control to 20.7 % (20.0% MPP). The polyphenol also increased from 540 to 4500 µg GAE/g increasing its scavenging activity (Ajila *et al.* 2008).

Composite brown flour was developed by enriching wheat bran up to 20 per cent. It was observed that as addition of wheat bran increased the iron content in the composite flour increased from 16.8 per cent (residual flour) to 29.2 per cent (flour with 20% wheat bran). Phytic acid content also increased from 0.72 (residual flour) to 1.09 g/100g in composite flour with 20 per cent wheat bran. Fiber (%) was highest in bran enriched composite flour. This composite flour was used to prepare different variations of chapatis and it was observed that as the bran per cent increased the fiber, phytic acid, iron and protin content increased (Butt *et al.* 2004).

Designer fibre enriched muffins and buns were developed replacing maida flour with composite flour prepared by adding malted cassava flour, chickpea flour, whole wheat flour, wheat bran (WB), rice bran (RB) and popped rice flour. The muffins and buns prepared by addition of wheat and rice bran had lower starch and sugar levels. The *in vitro* starch digestibility also decreased with addition of WB, RB. The total dietary fibre levels also increase in muffins and biscuits enriched with rice bran and wheat bran (Jisha *et al.* 2010).

Increased antioxidant activity and total dietary fibre was noted in breads enriched with defatted rice bran compared to control. Total dietary fibre in breads with 10 per cent rice bran was 10.91 ± 0.07 g/100 g whereas control had only 6.31± 0.02 (Sairam *et al.* 2011).

Breads prepared with rice, wheat and barley bran replacement of wheat flour at 10, 20, 30 per cent resulted in increased antioxidants activity. Total phenol content increased in bran enriched bread compared to control. Highest antioxidant activity was found in rice bran enriched bread followed by barley bran and wheat bran. 10 % bran enriched bread was acceptable and 5 alcohols, 6 pyrazines, 2 acids, 9 aldehydes, 5 ketones, 3 esters, and 6 sulphur-containing volatile compounds were identified in the bread. Higher content of alcohol was found in rice bran bread indicating one of the reasons for its low sensory score as some alcohols adversely affect acceptability of breads. Wheat bran bread had higher aldehydes compared to any other bread (Hussein and Ibrahim, 2019).

Buns prepared with addition of soy fibre (4, 6, 8 %) had higher nutritional content compared to control buns in terms of ash, dietary fibre, iron and calcium. The content for phytic acid and tannins also increased in fibre enriched buns compared to control. *In vitro* starch digestibility significantly decreased in fibre rich buns compared to control buns. *In vitro* bioaccessiblity for iron and calcium also increased in treated samples compared to control (Bhavya and Prakash, 2018).

Increased nutrient content was noted after addition of defatted apple seed cake at 5 per cent and 20 per cent in breads compared to control. The insoluble dietary fiber and prietin increased significantly with 20 per cent apple seed addition (Purić *et al.* 2020).

Muffins enriched with date syrup and wheat bran at various proportions viz., T0 (30:0), T1 (30:2.5), T2 (30:5), T3 (40:2.5), T4 (40:5), T5 (50:2.5) and T6 (50:5) were developed and it was observed that 2.5% wheat bran + 50% Date syrup was the best accepted formulation amongst all treatments. Nutrient analysis showed an increasing trend in calcium, manganese, zinc and iron values from T0 to T6 by a range of (108.63 to 111.14 mg/100 g), (0.29 to 0.43 mg/100 g), (1.39 to 2.37 mg/100 g) and (2.37 to 2.99 mg/100 g) respectively. This increase was due to increasing concentration of date syrup and wheat bran. Increasing trend was also noted in crude protein and crude fibre from 11.4 and 1.33 in T0 to 13.05 and 5.63 in T6 respectively (Yaseen *et al.* 2012).

Romjaun and Prakash (2013) developed fibre enriched muffins by addition of carrot powder, whole Bengal gram and wheat bran and it was revealed that control was best accepted followed by wheat bran muffins and carrot muffins. Moisture content of muffins ranged from 11.45 to 15.0 %, fat ranged from 13.46 to 15.40 %. Mineral content increased with addition of carrot powder and wheat bran as compared to control. Calcium, iron and phosphorous content increased from 30 mg /100 g, 2.06 mg/100g, 112 mg/100 g in control to 39.6 mg/100 g, 3.56 mg/100 g, 215 mg/100 g respectively in 24 per cent wheat bran incorporated muffins. Soluble (3.40 g/100 g) and insoluble (6.76 g/100 g) dietary fibre content was highest in 24 per cent wheat bran muffins.

Muffins prepared with incorporation of wheat grass resulted in increased of protein from 14.37 to 16.31 per cent, ash (1.21 to 2.31 %) and total dietary fibre from 2.22 to 6.31 per cent. Fat and carbohydrates content decreased as the amount of wheat grass increased (Rahman *et al.* 2015).

Antioxidant capacity of muffins enriched with cabbage outer leaf powder (KBP) revealed that increased addition of KBP increased the DPPH activity from 24.87 in control to 37.56 in 4 per cent KBP. There was increase moisture (20.38 to 23.90 %), protein (6.68 to 7.09 %), fat (14.83 to 15.33 %), ash (1.13 to 1.94 %) and dietary fibre (6.71 to 12.73 %) in control to 4 per cent KBP. Decrease was observed in carbohydrate content and energy Heo *et al.* (2019).

Nithya *et al.* (2013) studied antioxidant activity of rice bran enriched pasta using DPPH and reducing power assay and revelaed that antioxidant activity increased significantly in rice bran enriched pasta as percent incorporation increased. Highest radical scavenging activity, redcuing power and total phenolic compounds were noted in 20 per cent bran enriched pasta compared to pasta with 5 per cent bran.

Extruded snacks prepared by addition of red, white and purple wheat bran obtained from various varieties viz., hard red spring, soft white club cv. Bruehl, and purple wheat at 12.5, 25 and 37.5 per cent in waxy wheat flour for preparation of extruded snacks resulted in increased antioxidant capacity. It was observed that higher the pigmentation and higher the concentration greater was antioxidant capacity expressed as Trolox equivalents (TE). Thus, purple and red wheat bran incorporation had higher antioxidant capacity compared to control and white wheat bran (Fleischman *et al.* 2016).

In a study conducted by Guo *et al.* (2018) to produce antioxidant rich wine from foxtail millet bran, it was seen that foxtail millet bran wine (MBW) had higher antioxidant potential having six times higher polyphenol content compared to millet wine (MW) coupled with stronger antioxidant activity in DPPH, TEAC and FRAP assays. Four phenolics were identified vanillic acid, syringic acid, pcoumaric acid, and ferulic acid. Amino acids were also higher in MBW compared to MW and commercially available millet wine (CAMW). It was concluded that millet bran wine could have possible application as functional beverage, nutraceutical or dietary supplement.

A randomized, double blind, noncross-over study conducted for 6 weeks on three groups of healthy individuals revealed that rice bran and oat bran should be used as part of diet for treatment of hyperlipidaemia. Subjects were divided in three groups and were given 84 g of products either rice bran, oat bran or rice starch in addition to their regular prudent diet. Inclusion of rice bran and oat bran resulted in reduction of Serum cholesterol, Serum apoB. No significant difference was observed in HDL, triglycerides and apoA. Ratio of LDL-C: HDL-C was decreased significantly. The decrease in LDL was 13.7 ± 2.8 per cent in the rice bran group and 17.1 ± 2.4 per cent in the oat bran group ($p \leq 0.05$) (Gerhardt and Gallo, 1998).

In pasta incorporated with inulin and guar fibre overall glucose release was reduced which is partly associated with reduction of starch due to addition of fibre. Significant decrease ($p < 0.001$) in glucose release was observed in pasta containing guar gum at 90 min (Tudoricaÿ *et al.* 2002).

No significant difference was observed in markers of glycaemic index and risk factors of coronary heart disease (CHD) such as weight, fasting blood glucose, HbA1c, serum lipids, apolipoproteins, blood pressure, serum uric acid, clotting factors, homocysteine, C-reactive protein, magnesium, calcium, iron, or ferritin in test or control group receiving cereal fibre (wheat bran) viz., 19g and 4g per day respectively for 3 months. The fibre was included in subjects' diet via high-fibre breakfast cereals and bread. Authors concluded that longer duration studies may be required to demonstrate the benefits of cereal fibre (Jenkins *et al.* 2002).

Jowar bran, wheat bran and mixed bran (jowar + wheat bran) was used to develop *papdi*. These *papdis* were supplemented to 45 non-insulin dependent, non-obese 45 diabetes subjects of Kolhapur district within age group 40-50 years. These patients were divided in 3 main groups based on grades hyperglycemia of viz., 1st group >120-170, 2nd group 171-221 and 3rd group >221 mg/100 ml. Further the groups were subdivided into jowar bran, wheat bran and mixed bran groups. Supplementation of bran *papdis* gradually increased each month to provide 10, 20, 30 g bran at 1st, 2nd and 3rd month respectively. The results indicated that jowar, wheat and mixed bran could significantly lower the blood glucose levels in all the 3 main groups (Kamble and Shinde, 2004).

Oat bran enriched fresh muffins (8 g, 12 g β-glucan per serving) when supplemented fresh to 11 subjects significantly reduced the peak blood glucose rise (PBGR) compared to muffins (8 g, 12 g β-glucan per serving) treated with 2, 4 freeze thaw cycles. It was observed that freeze-thaw cycles affected the solubility of β-glucan thus reducing its effect on lowering the post prandial blood glucose response (Lan-Pidhainy *et al.* 2007).

Glycaemic index of untreated and oat bran muffin treated with or without β-glucanase was analysed in 10 healthy subjects and area under curve (AUC), peak blood glucose rise (PBGR) was lower in oat bran muffins with 8 g and 4 g serving of β-glucan compared to control wheat muffins (Tosh *et al.* 2008).

In a crossover study plasma and urine were analysed for total phenolics (TP) and antioxidant potential (AP) using FRAP for 17 subjects at baseline and after giving wheat bran, rice (93 g approximately). It was observed that TP and AP increased significantly after 1, 3 hour in plasma respectively after wheat bran consumption. Urine samples also showed significalnty higher TP and AP values after wheat bran consumption. Authors suggested that wheat bran phenolics can improve the antioxidant status after consumption (Price *et al.* 2008).

A study conducted on 10 healthy male individuals the glucose responses for sorghum muffin significantly lowered 45, 60, 75, 90, and 120 minute intervals ($P < 0.05$) compared to wheat muffins. The mean incremental area under the curve (iAUC) significantly lowered plasma glucose responses about an average of 35% for sorghum muffins (Poquette *et al.* 2014).

2.8 Shelf life study of designer products incorporated with millet and cereal bran

Bakery products viz., cake, breads, muffins, buns have shorter shelf life of 4-10 days compared to biscuits, rusks etc. due to the nature of ingredients used (milk, eggs) and higher moisture content making them susceptible to microbial spoilage with moulds being most expensive and grave problem (Doulia *et al.* 2006 and Saranraj and Geetha, 2012). Thus, addition of preservatives, good packaging conditions becomes important for improving the shelf life of such products.

The shelf life studies of defatted rice bran cookies for 60 days revealed that during storage the sensory acceptability decreased (Sharif *et al.* 2009).

Full fat and defatted wheat, rice, corn, oat and barley bran were used in biscuit preparation. It was observed that the sensory palatability decreased with increasing storage period but it was high till 3 months. Microbial count also increased but stayed within the permissible standards till 3 month storage period. Increase in FFA was also noted during storage period (Nagi *et al.* 2012).

Cookies formulated using 25, 50, 75 and 100 per cent rice bran were studied for 30 days of storage by conducting microbial analysis. No microbial contamination was observed during the storage period (de Souza *et al.* 2019).

In study conducted by Ayub *et al.* (2003) it was observed that moisture content of bread samples increased with increase in storage period of 4 days. Microbial colonies were recorded from 3rd day of storage, and as the storage period increased there was significant increase in microbial population. Sensory scores for also significantly decreased over the storage period.

Kamaljit *et al.* (2011) studied shelf life of high fibre breads incorporated with psyllium husk and oat fibre on bases of organoleptic properties and visual spoilage of mould growth. It was observed that refrigerated breads had no visual spoilage till 7th day whereas spoilage occurred at 5th day for breads stored at room temperature.

Bhise and Kaur (2014) stored breads prepared with glycerol, sorbitol and mannitol (0–6 %) at ambient (30±1 °C) and refrigerated (4–6 °C) conditions for 10 days period. The breads were assessed for sensory parameters, moisture, water activity and free fatty acid (FFA) during storage. Water activity and FFA increased during storage and was highest on 10th day. As the storage period increased acceptability of product decreased. It was observed that addition of polyols increased the shelf life from 4 days to 10 days.

Breads prepared with rice wheat at barley bran with 10, 20, 30 per cent replacement of wheat flour were tested for freshness and staling for 7 days at room temperature using alkaline water retention capacity (AWRC) test. No changes were observed in first 3 days, but the rate of staling increased by 7 days of storage. The bran incorporated breads were fresher than control during the storage period. This may have been due to high fibre in the bran enriched breads (Hussein and Ibrahim, 2019).

Fibre enriched muffins prepared with addition of wheat bran at 8, 16, 24 per cent were stored at room temperature for 7 days and FFA, peroxide values (PV) were analysed. Addition of wheat bran at 24 per cent resulted in higher FFA values on initial day compared to control. FFA and PV increased at 7th day of storage. This indicated that there was decrease in quality of muffins during 7 days storage at room temperature (Romjaun and Prakash, 2013).

Storage study of extruded snacks enriched with rice, oat and wheat bran showed that moisture, FFA and water activity increased by the end of 6 months storage periods. Decrease was also noted in total phenolic content, Trolox equivalent antioxidant capacity, DPPH activity. Total plate count (cfu/g) of rice bran enriched snack was more than wheat or oat bran enriched snacks. Though increase was noted in total plate count, it was well within standard linmits (Dar *et al*. 2016).

Chapter III

MATERIAL AND METHODS

The present work titled "Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential" aimed at value adding buns and muffins with Kodo millet (*Paspalum scrobiculatum*), Proso millet *(Panicum miliaceum)*, Barnyard millet *(Echinochloa esculenta)* and Foxtail millet (*Setaria italica)* brans. The study was conducted in Department of Foods and Nutrition, Post Graduate Research Centre, Millet Processing and Incubation Centre, Central Instrumentation Cell and Quality Control Laboratory, Professor Jayashankar Telangana State Agricultural University. This chapter gives entire details of methodology followed in the study under following sub headings.

3.1 Procurement of raw material

3.2 Dehulling of minor millets for bran extraction

3.3 Stabilization of minor millet brans

3.4 Formulation and development of designer products incorporating millet brans

3.5 Sensory evaluation of designer products

3.6 Phytonutrient analysis of brans and best accepted products

3.7 Nutrient and antioxidant analysis of bran and best accepted products

3.8 Physical and functional properties of minor millet brans, bran blended flours and designer products

3.9 Shelf life of best accepted minor millet bran incorporated designer products

3.10 Therapeutic potential of best accepted minor millet bran incorporated designer products

3.11 Statistical analysis

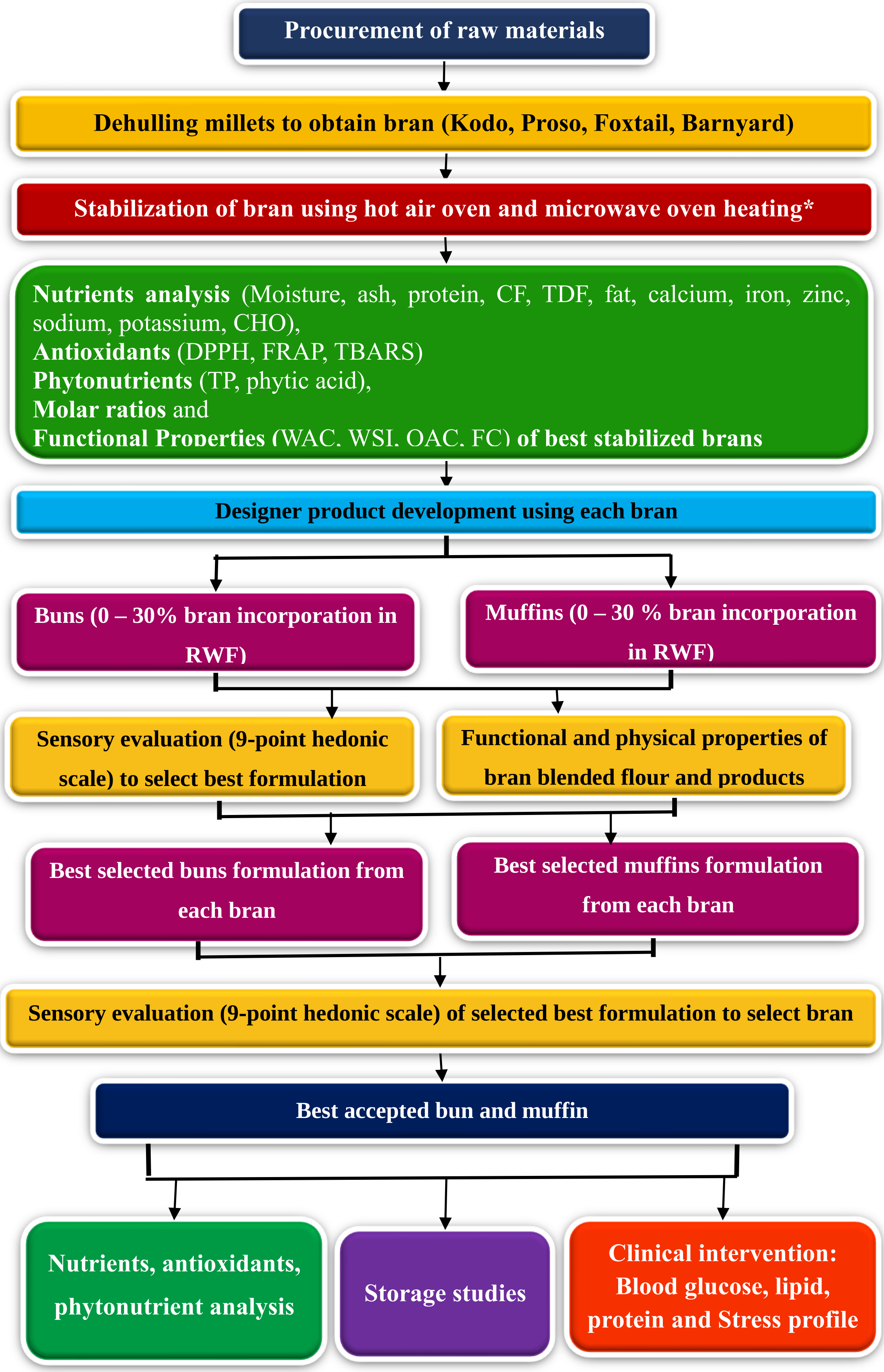


Fig 3.1 Research Design

Note: *stabilization is further explained in detail in Fig 3.2

3.1 Procurement of raw material

Bulk market sample of whole millets (kodo millet, proso millet, barnyard millet and foxtail millet) were directly procured from farmer from Tamil Nadu. Other raw materials required for product development were procured from local market. All the required chemicals of standard laboratory grade were procured from standard suppliers.

3.2 Dehulling of minor millets for bran extraction

Commercially like rice and wheat bran, millet brans are not collected, but are discarded or used as animal feed. For research purpose various authors have reported bran / hull collection from millets by dehusking, dehulling, polishing or milling grains. Use of barley pearlers (Lorenz and Dilsaver, 1980), dehullers like abrasive disc dehullers (Gaitan *et al.* 1989; Sikwese and Duodu, 2007; Herald *et al.* 2012 and Rhodes, 2014), rice milling machine (Kumari *et al.* 2017a), pulverisers like plate mill, local flour mill (Sridevi *et al.* 2011; Suma and Urooj, 2012; Shan *et al.* 2014; Nazni and Karuna, 2016 and Palaniappan *et al.* 2017), centrifugal sheller (Devisetti *et al.* 2014), polisher and grain dehusker (Bora *et al.* 2018) were reported. Considering all the previous methods used, in the present study bran was collected after dehulling the grains using abrasive dehuller (Gurunanak Engineering) in Millet Processing and Incubation Center, PJTSAU.

All millets were cleaned to remove stones using destoner and grader (Sowbhagya Industries, Andhra Pradesh), followed by manual cleaning to remove any other foreign materials or twigs etc. The grains were dehulled using stone abrasive dehulling machine (Gurunanak Engineering) for 30 minutes for proso, foxtail, barnyard millet and 35 minutes for kodo millet (Plate 3.1). Kodo millet bran (KB), proso millet bran (PB), foxtail millet bran (FB) and barnyard millet bran (BB) were obtained by dehulling kodo millet, proso millet, foxtail millet and barnyard millet respectively. Dehulled grains were sieved and winnowed to separate bran. The bran was collected and stored at –20 ºC (Vestfrost Freezer) to avert lipolytic activities till sample preparation for executing stabilization treatments.

Operational Definition of Bran: The material obtained after dehulling whole grain apart from the dehulled grain was considered as bran. It included outer coating, some part of husk, some powdered grain crushed during dehulling and bran fractions.

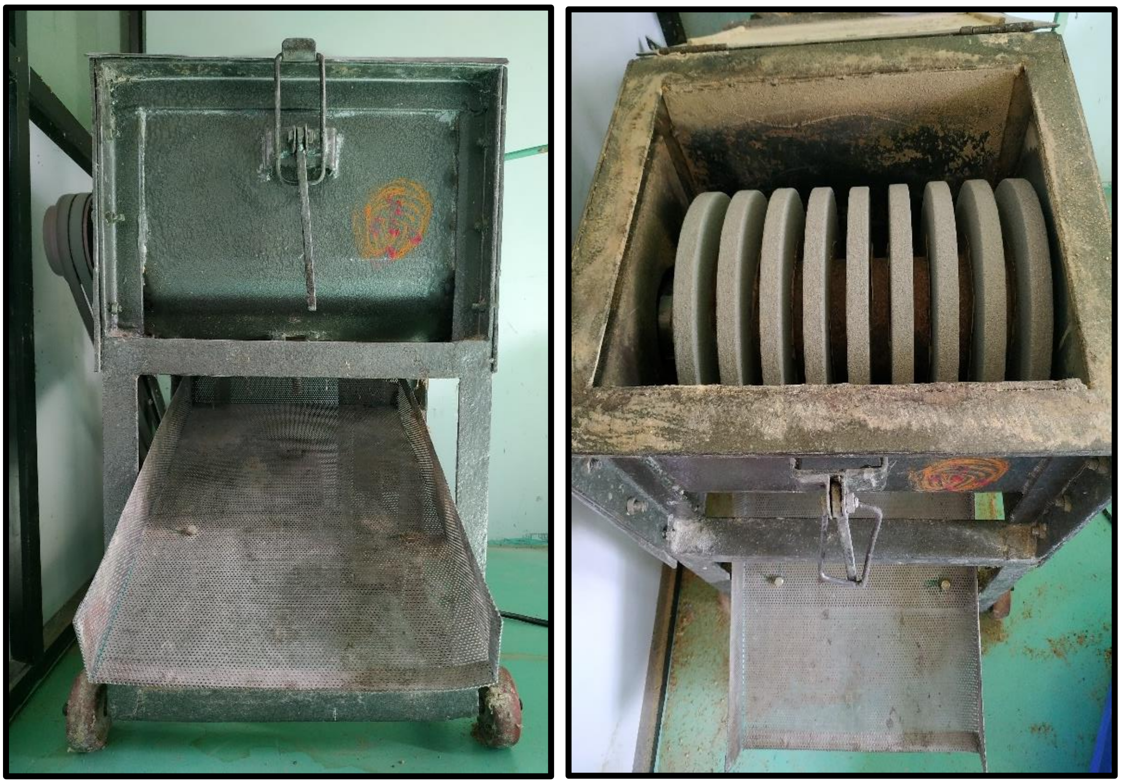


Plate 3.1 Stone abrasive dehuller

3.2.1 Percentage of bran recovery, dehulling per cent and degree of dehulling (Gautam *et al.* 2008)

Protocol described by Gautam *et al.* (2008) was used with modification. The amount of bran (true bran + broken grain + husk) obtained was measured to calculate percentage of bran and the dehulled grain obtained was used to calculate dehulling percentage. Degree of dehulling was calculated using the formula described by Sreerama *et al.* (2009). Plate 3.2 shows whole millet grains and bran obtained.

$$\text{Percentage of bran recovered} = \frac{\text{Weight of bran obtained}}{\text{Weight of initial sample taken}} \times 100$$

$$\text{Dehulling \%} = \frac{\text{Weight of dehulled grain}}{\text{Weight of initial sample taken}} \times 100$$

$$\text{Degree of dehulling (\%)} = \frac{\text{Weight of dehulled grain} - \text{Weight of un dehulled grain}}{\text{Weight of initial sample taken}} \times 100$$

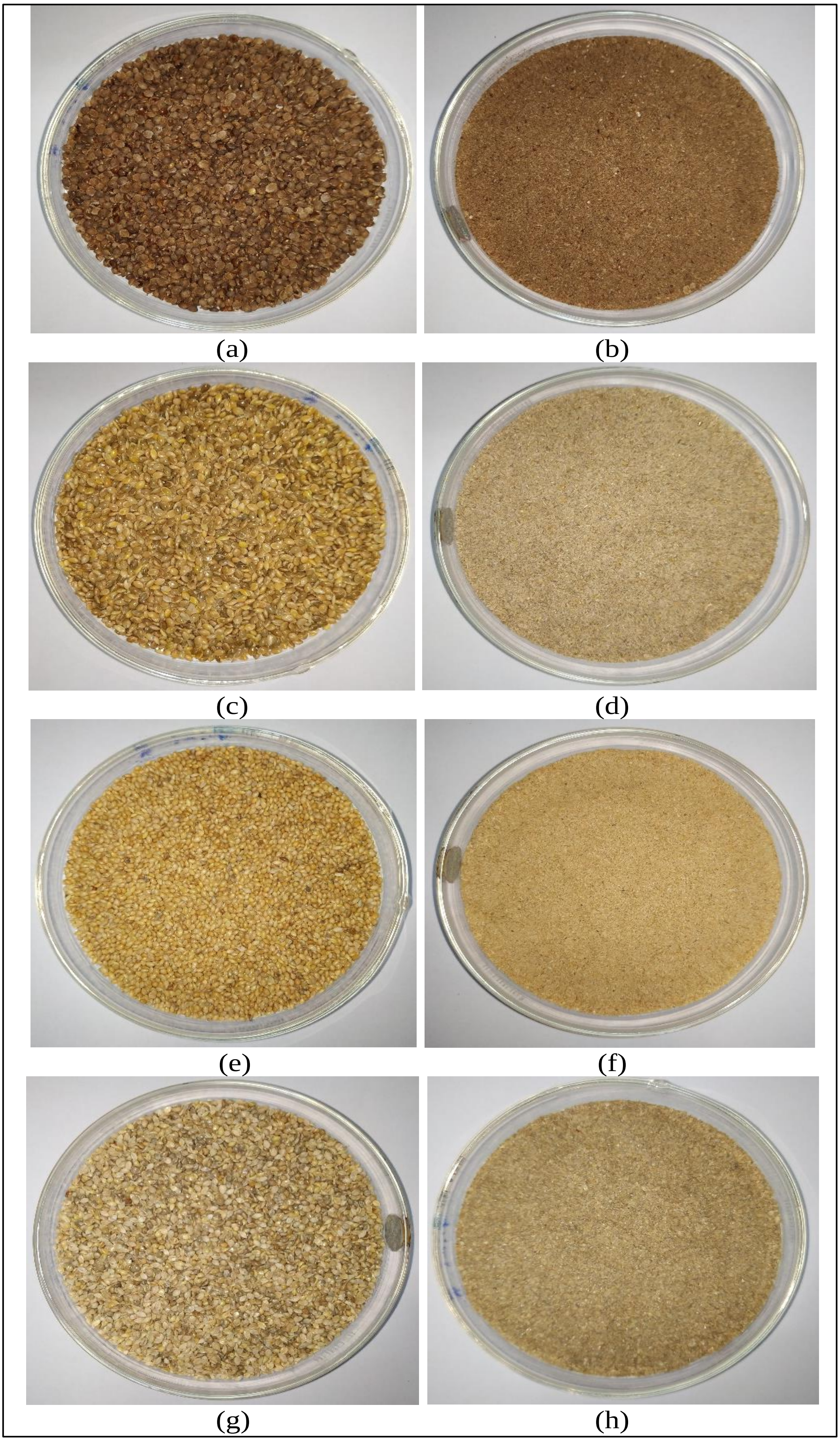
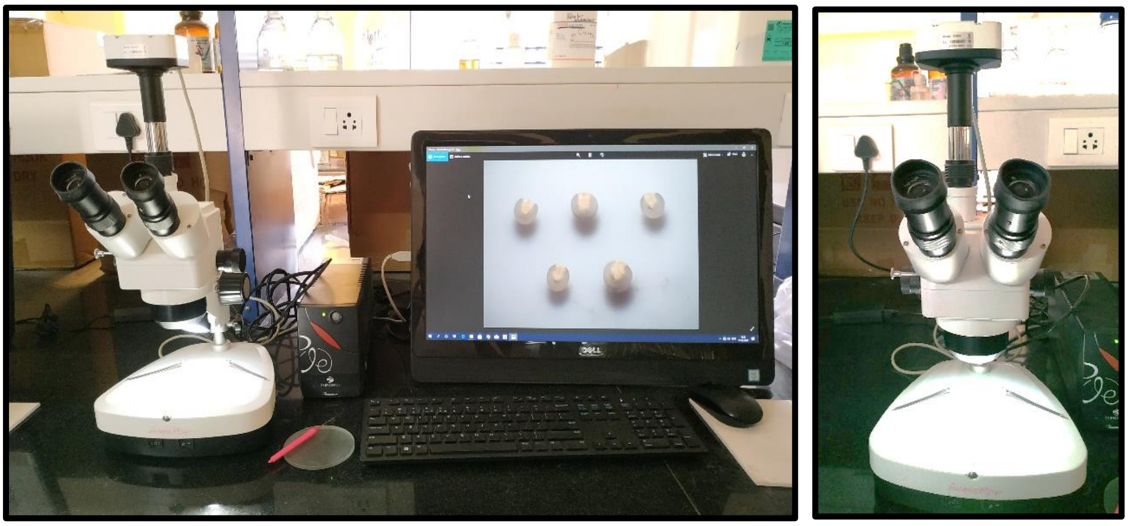


Plate 3.2 Whole millet grain (kodo (a), proso (c), foxtail (e), barnyard (f)) and their brans (kodo (b), proso (d), foxtail (f), barnyard (h))

3.2.2 Grain dimensions and digital image capture (Espinosa-Mendoza *et al.* 2012)

Grain dimensions such as length, width and perimeter were measured for both whole grain (before dehulling) and dehulled grain (after dehulling). Commonly calipers are used for measuring grain dimensions but considering the limitations such as time taken, chances of human error, accuracy etc., image analysis is a better alternative. It gives a clear, to scale photographic evidence. Reduces time taken for measuring, human errors and ensures accuracy (Tanabata *et al*. 2012).

Thus, in the present study digital image capture method was used with slight modifications as described by Espinosa-Mendoza *et al.* (2012). For this, 10 grains were randomly selected from each grain samples viz., kodo, proso, foxtail and barnyard millet (whole and dehulled). The grains were observed under stereozoom microscope (Make: Lawrence and Mayo) connected to computer installed with TC capture software at 1x magnification and high-resolution images were captured using camera attached to microscope body (Plate 3.3). Further grain dimensions from captured image were measured for length, width and perimeter using scale set for 1x magnification from the calibration table (Plate 3.4). Average of 10 grains was reported for grain dimensions of length, width and perimeter.



(a) (b)

Plate 3.3 Stereo zoom microscope with computer (a), stereo zoom microscope (b)

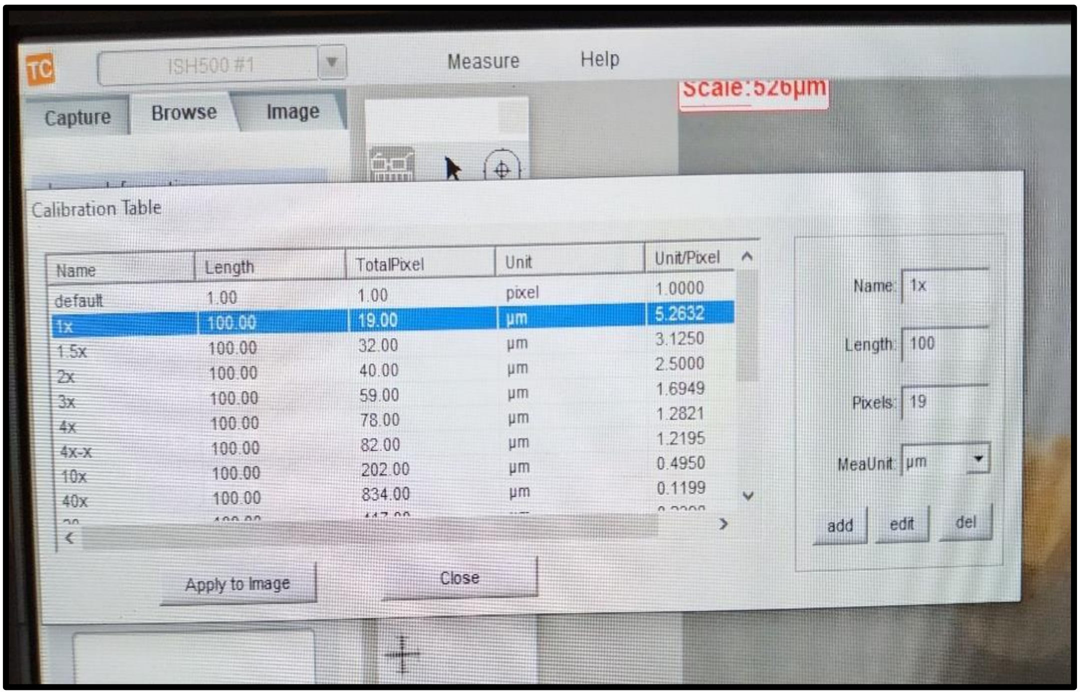


Plate 3.4 TC capture software calibration table

3.3 Stabilization of minor millet brans

Stabilization process is essential to improve shelf life of bran by inactivating lipolytic enzymes. There is limited research conducted on stabilization of the minor millet bran thus, reference treatments utilized for rice and wheat bran stabilization were selected and suitable protocols with slight modification were used in the present study. Minor millet brans were stabilized using four treatments viz.,

HT1: Drying in hot air oven at 100 ºC for 3 hours (Sudha *et al.* 2011),

HT2: Drying in hot air oven at 130 ºC for 20 minutes (Bagchi *et al.* 2014),

MW1: Heating in microwave oven for 1.5 minutes at 900 W (Bagchi *et al.* 2014) and

MW2: Heating in microwave oven for 2.5 minutes at 900 W (Bagchi *et al.* 2014).

Moisture and Free fatty acids (FFA) were estimated in the bran during storage period of 15 days to select best stabilization treatment (Fig 3.2). Best treatment of bran stabilization was continued further for nutritional, antioxidant, phytonutrient /anti-nutritional analysis and product development.

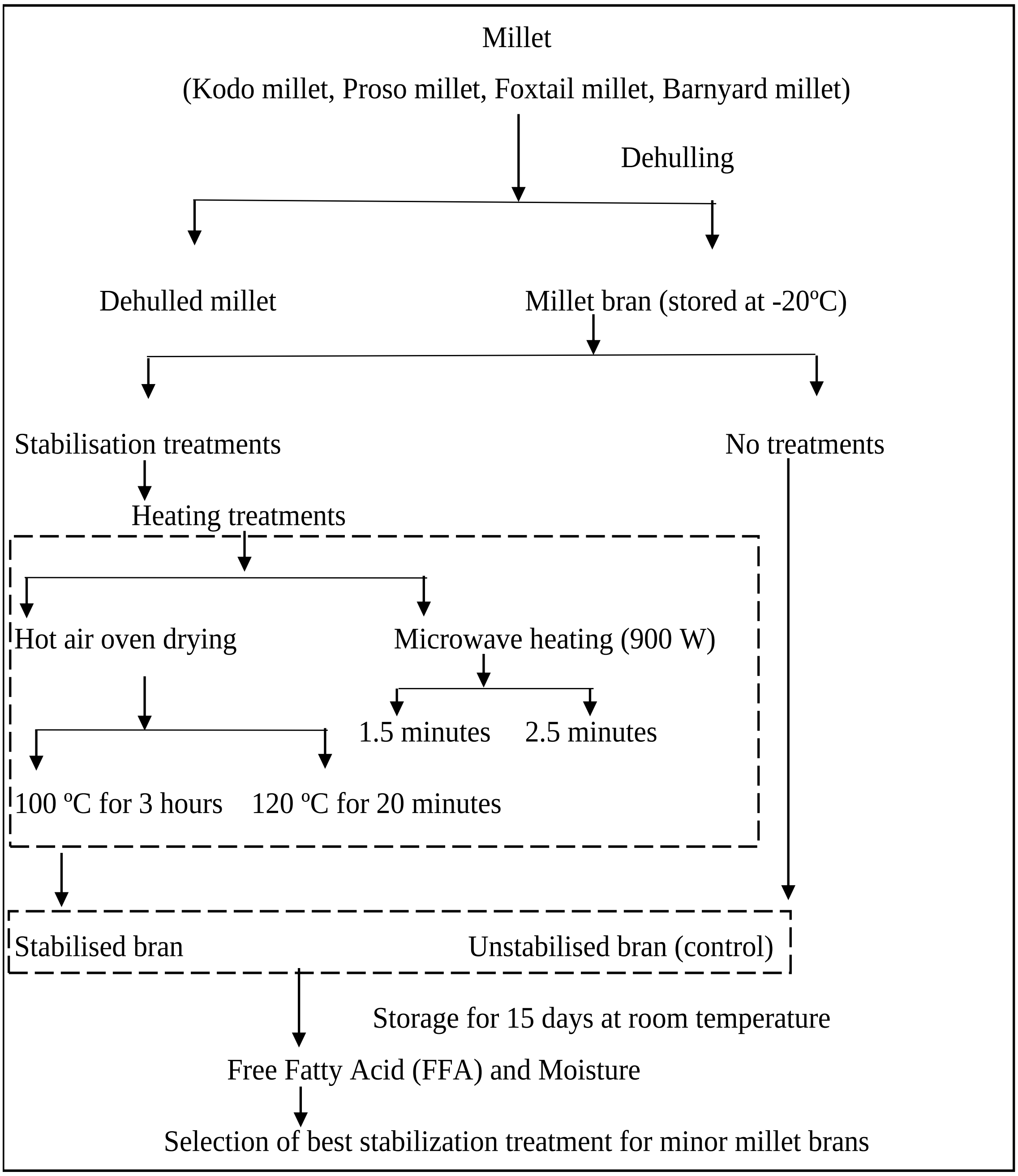


Fig 3.2 Schematic diagram for millet bran stabilisation

3.3.1 Estimation of moisture (IS:1155 – 1968 reaffirmed 2010)

Moisture estimation was done by weighing 5 g of sample in pre weighed Petri dish. All bran samples were dried at 130 ± 3 °C for 2 hours. After 2 hours Petri dishes were removed, cooled in desiccator and weighed. The process of drying, cooling and weighing was repeated at half hour intervals till constant weight was achieved and the difference between two consecutive weights was less than 1 mg. Each time before weighing, the Petri dishes were cooled in desiccator. Moisture content of the sample was expressed in g/100 g of sample.

3.3.2 Estimation of free fatty acid (AOAC 940.28 – 2010)

Free fatty acids formed due to effect of moisture, temperature and lipase activity are measure of rancidity during storage. Free fatty acids were estimated at 0, 7th and 15th day to check the stability of bran treated with various stabilization treatments in comparison to control (untreated). Standard protocol (AOAC 940.28; Sadasivam and Manickam, 2007 and Bagchi *et al.* 2014) with slight modification was used to determine free fatty acids. Soxhlet apparatus was used to extract oil from millet brans for conducting the experiment. Accurately weighed oil was dissolved in ethyl alcohol (10 ml) and few drops of phenolphthalein (1 %) was added as an indicator. This solution was titrated with vigorous shaking against 0.1 N NaOH solution. Free fatty acid was calculated using formula:

$$\% \text{ FFA (as oleic acid)} = \frac{(V \times N \times 28.2)}{M}$$

Where, V = volume of NaOH (ml) required for titration, N = normality of NaOH and m = accurate weight of oil (g).

3.4 Formulation and development of designer products incorporating millet brans

Two products *i.e.,* buns and muffins were developed incorporating different millet bran (Foxtail, Barnyard, Kodo, Proso millet bran) in varying proportions replacing the refined wheat flour at 0, 10, 15, 20, 25, 30 per cent (Table 3.1). These products were selected for incorporation of millet bran as they are consumed on a large scale in all sections of population and are also one of the empty calorie baked goods. Thus, enhancing the nutrient profile of such products is need of the hour. Also, it’s a challenge to incorporate bran in bakery products thus in the present study various formulations were tried and tested. Plate 3.5 shows pre-preparation of muffins and buns.

3.4.1 Formulation of Muffins

Muffins were prepared by standardizing the recipe with modifications as described by (Heo *et al.* 2019). Six variations were prepared with each bran (Table 3.2). Refined wheat flour was replaced with 0 to 30 per cent stabilized millet bran. The flour, baking powder and salt were mixed thoroughly and sifted thrice using domestic sieve. In a vessel, oil and sugar were creamed until fluffy to which egg was added and creamed again until it became light and fluffy. To this flour was added slowly in batches and mixed nicely. The batter was poured in muffin tray and baked at 180 °C for 25 min in a preheated oven (Karr Engineering).

Table 3.1. Formulation used for preparation of designer muffins and buns

Sl. No	Millet Brans	Level of bran incorporation (%)					
		0	10	15	20	25	30
1.	Foxtail Millet	RWF	FSM10	FSM 15	FSM 20	FSM 25	FSM 30
2.	Barnyard Millet	RWF	BSM10	BSM15	BSM20	BSM25	BSM30
3.	Proso Millet	RWF	PSM10	PSM 15	PSM 20	PSM25	PSM 30
4.	Kodo Millet	RWF	KSM10	KSM15	KSM20	KSM25	KSM30

Note: RWF: Refined wheat flour

Table 3.2. Standardized muffins recipe

Ingredients/Variations	M1 (0 %)	M 2 (10 %)	M 3 (15 %)	M 4 (20 %)	M 5 (25 %)	M 6 (30 %)
Refined Wheat Flour (g)	50	45	42.5	40	37.5	35
Bran (g)	0	5	7.5	10	12.5	15
Sugar (g)	40	40	40	40	40	40
Oil (ml)	20	20	20	20	20	20
Egg (g)	38	38	38	38	38	38
Milk (ml)	30	30	30	30	30	30
Vanilla essence (ml)	2	2	2	2	2	2
Baking powder (g)	2	2	2	2	2	2
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5

M= Muffins

3.4.2 Formulation of buns

Buns were prepared by standardizing with some modifications, the recipe described by Arora and Saini (2016). Ingredients viz., flour (100 g), salt (1 g), sugar (15 g), fat (10 g) were weighed in a container (Table 3.3). Flour was sifted thrice using domestic sieve. Yeast (2.5 g) was weighed in a container to which 60 ml of lukewarm water was added and kept aside for 15 mins. Dough was made using yeast water and mixing all ingredients properly. Kneading was done for 10-15 mins till good consistency was obtained. Prepared dough was then brushed with oil and proofed for 1 hour, till it became double its volume. After proofing dough was divided into two equal portions and made into smooth round balls. These balls were places on greased baking tray, bun rings were kept around the ball to ensure proper round shape and they were allowed to stand for 15 mins before baking. Buns were baked at 180 °C for 25 minutes in preheated oven

(Karr Engineering). Six variations were prepared with each bran by replacing refined wheat flour with 0 to 30 per cent stabilized millet bran.

Table 3.3. Standardized buns recipe

Ingredients/Variations	**B 1 (0 %)**	**B 2 (10 %)**	**B 3 (15 %)**	**B 4 (20 %)**	**B 5 (25 %)**	**B 6 (30 %)**
Refined Wheat Flour (g)	100	90	85	80	75	70
Bran (g)	0	10	15	20	25	30
Sugar (g)	15	15	15	15	15	15
Fat (g)	10	10	10	10	10	10
Yeast (g)	2.5	2.5	2.5	2.5	2.5	2.5
Salt (g)	1	1	1	1	1	1

B= Buns

3.5 Sensory evaluation of designer products (Peryam and Pilgrim, 1957)

Sensory evaluation of the products was conducted at two levels using nine-point hedonic scale (Appendix A) by semi-trained panel (N=21). The panelists were asked to score products for appearance colour, texture, flavour, taste and overall acceptability. The scores ranged from 1 = dislike extremely to 9 = like extremely. First level was conducted to obtain best accepted muffins and buns within each bran variation (10 – 30 %). Further the best accepted muffins and buns from each bran variation were again subjected to second level sensory evaluation for final selection of one bran among best accepted muffins and buns to be used in supplementation studies. It was conducted in a special laboratory designed for sensory evaluation at Post Graduate and Research Center, PJTSAU (Plate 3.6). Panelists were provided with glass of water to rinse their mouth in between evaluating each sample to neutralize the taste of previous sample.

3.6 Phytonutrient analysis of brans and best accepted products

The stabilized millet brans were stored at – 20 ºC until further tested for phytonutrients viz., total phenols and phytic acid as it is reported to be a good source of phenolic compounds and phytic acid. Best accepted products were also estimated for their phytonutrients.

(a) (b) (c)

Plate 3.5 Pre-preparation of muffins (a) buns (b) and baking in rotary oven (c)

(a) (b)

(c)

Plate 3.6 Sensory evaluation of muffins (a) and buns (b) in laboratory (c)

3.6.1 Methanolic extraction of sample for total phenol and antioxidant estimation

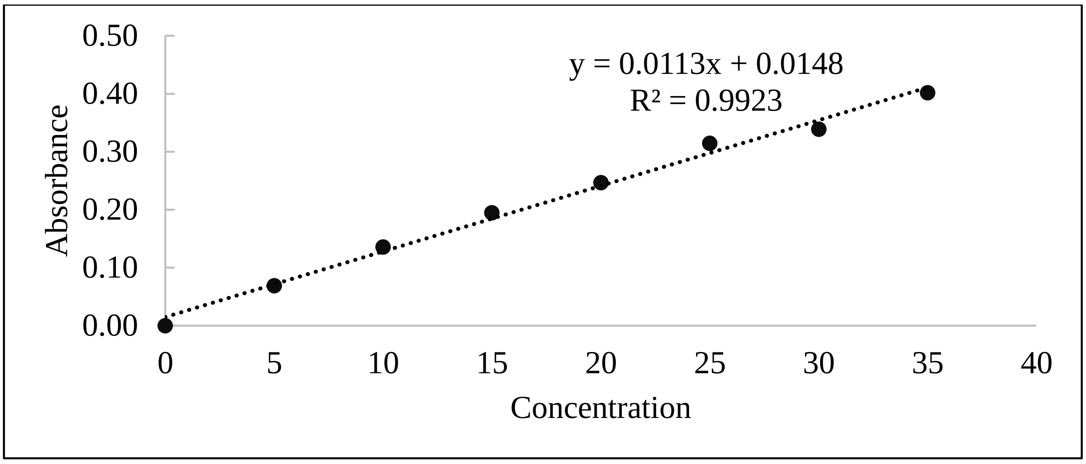
Previous studies have reported use of acetone (Chandrasekara and Shahidi, 2011), methanol (Singh *et al.* 2002), ethanol (Amadou *et al.* 2011) and combinations of these solvents with water (Kumari *et al.* 2017a) for extraction of samples but it was observed that methanol was cheap and efficient in extraction (Suma and Urooj, 2014). Thus, in the present study methanolic extractions of sample was done.

In a conical flask 0.5 g of millet bran sample or 1 g of muffin/bun sample was weighed and 15 ml of 80 per cent methanol acidified to pH 2.0 was added. The mixture was extracted by continuous shaking at room temperature for 30 min. Supernatant was collected and the residue was re-extracted twice. All the three supernatants were collected centrifuged at 6000 rpm for 15 min and filtered using Whatman No. 1 filter paper. Volume of the extract was noted and made to 50 ml using solvent. The extracts were transferred to micro centrifuge tubes and stored at – 20 °C till further use.

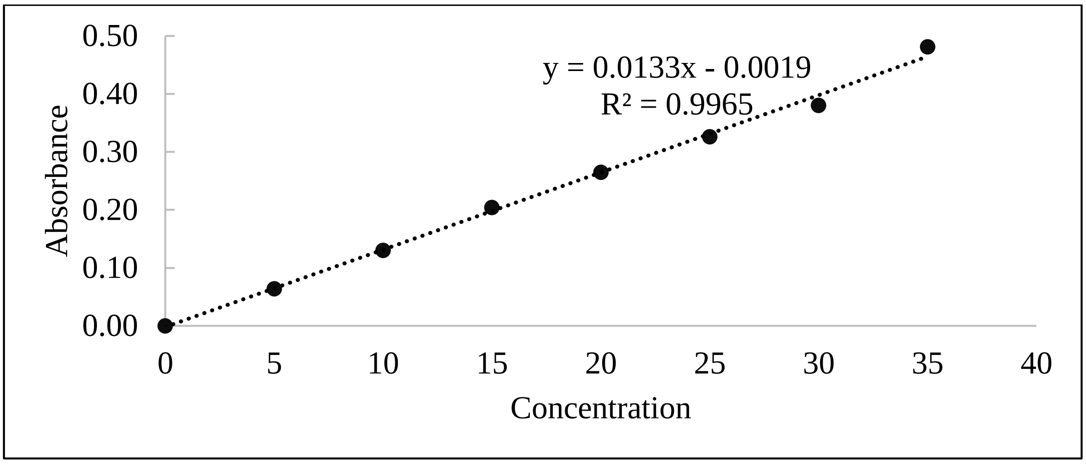
3.6.2 Estimation of total phenol (Slinkard and Slingleton, 1997)

The Folin–Ciocalteu (FC) method (Slinkard and Slingleton, 1997) as described by Sadasivam and Manickam (2007) was used with slight modification for estimation of total phenol content. In alkaline medium the phenols react with FC reagent to form blue colour complex and this can be measured spectrophotometrically. Sample aliquot of 0.1 ml (millet bran) or 0.2 ml (muffins/buns) was taken in a test tube and 0.5 ml of FC reagent diluted with distilled water (1:1 v/v) was added followed by addition of 10 ml of (7.5 %) sodium carbonate. This mixture was kept at 37 °C for 60 min and the absorbance was measured at 765 nm. Gallic acid was used to obtain the standard curve (5 µg-35 µg) and treated same way as sample. Total phenol content was determined from standard curve (Fig 3.3) prepared using different concentrations of gallic acid standard ($y = 0.0113x + 0.0148$; $R^2 = 0.992$ for brans and $y = 0.0133x - 0.0019$; $R^2 = 0.9965$ for products) and expressed as mg gallic acid equivalent (GAE)/100 g of the millet bran or best accepted products.

Calculation: TP (mg GAE/100g) = std. conc./std OD × sample OD/aliquot taken × volume made up/sample taken × 100/1000 × dilution factor



(a)



(b)

Fig 3.3 Standard curve for total phenols for bran samples (a) and products (b)

3.6.3 Estimation of phytic acid (Wheeler and Ferrel, 1971)

Estimation of phytic acid (Wheeler and Ferrel, 1971) for all brans and best accepted products was conducted as described by Sadasivam and Manickam (2007). Phytic acid is the storage form of phosphorous in seeds contributing to approximately 70 per cent of total phosphorous and it is considered as antinutritional factor due to its possible interference by forming complexes with essential nutrient during digestion and absorption especially of minerals, proteins and amino acids.

Principle: Trichloroacetic acid extracts phytates as precipitate of ferric salt. This iron from precipitate is estimated calorimetrically and the phytate phosphorous content is calculated using a constant 4Fe:6P molecular ratio in the precipitate.

Reagents:

1. 3 % Trichloroacetic acid (TCA)
2. 3 % Sodium sulphate in 3 % TCA

3. NaOH (1.5 N)
4. HNO_3 (3.2 N)
5. $FeCl_3$ solution prepared by dissolving 583 mg $FeCl_3$ in 100 ml 3 % TCA
6. Potassium thiocynate (1.5 M) prepared by dissolving 29.15 g in 200 ml distilled water.
7. Standard Fe $(NO_3)_3$

Preparation of standard: Standard was prepared by weighing 433 mg of $Fe(NO_3)_3$ and making up volume with distilled water to 100 ml in volumetric flask (Stock solution). Further working standard was prepared by taking 2.5 ml from stock solution and making up to 250 ml. From working standard 2.5, 5, 10, 15 ml of aliquot were taken in different 100 ml volumetric flask and diluted with little water, to which 20 ml of potassium thiocynate was added and volume was made up to 100 ml. All these standards were read immediately within a minute at 480 nm to create standard graph.

Extraction and sample preparation: Finely ground sample was weighed accurately. The sample taken was estimated to contain 0.5 to 30 mg P. Further the sample was extracted in 50 ml 3 per cent TCA for 30 mins with continuous shaking. Solution was then centrifuged and 10 ml supernatant was transferred into a 40 ml conical flask. To this 4 ml of $FeCl_3$ solution was added. The content in conical flask were further boiled on boiling water bath for 45 minutes, if the supernatant was not clear 2 – 3 drops of 3 per cent sodium sulphate was added. The suspension was centrifuged for 15 minutes and supernatant was decanted. The precipitate was washed twice by dispersing in 25 ml of 3 per cent TCA in boiling water bath for 10 min followed by centrifuging the content. sample was further washed with water. The precipitate was added with few ml of water and 3 ml of NaOH. Then the volume was made up to 30 ml and the sample was kept in boiling water bath for 30 mins. Further samples were filtered using Whatman filter paper No 2. The precipitate was washed with 70 ml of hot water and filtrate was discarded. The precipitate was dissolved in 40 ml of hot HNO_3 (3.2 N) in a 100 ml conical flask followed by washing the filter paper with distilled water and collecting these washing in the same conical flask and making the volume to 100 ml. From this 5 ml of aliquot were further taken for analysis.

Procedure: The aliquot taken from extracted sample (5ml) was transferred in 100 ml volumetric flask, diluted to approximately 70 ml to which 20 ml of potassium thiocynate was added. This solution was immediately read within a minute at 480 nm. A blank was run with each set of samples. Concentration of iron (µg) was calculated using standard graph (concentration = +12.5*A; R^2=1.0) and further was used to calculate phytate.

Calculation:

$$\text{Phytate (mg/100 g)} = \frac{\text{µg iron} \times 15}{\text{Weight of sample (g)}}$$

3.7 Nutrient and antioxidant analysis of bran and best accepted products

Best stabilized kodo, proso, foxtail and barnyard millet bran samples and best accepted products were analyzed for their nutrient content. All analyses were carried out in triplicates using standard protocols.

3.7.1 Estimation of moisture (IS:1155 – 1968 reaffirmed 2010)

Moisture estimation was done by weighing 5 g of sample in pre weighed Petri dish. The bran samples were dried at 130 ± 3°C for 2 hours whereas the muffin and bun samples at 105 ± 2°C in a hot air oven (Ria Instruments, Hyderabad) for 4 hours. After 2 hours the Petri dish was removed, cooled in desiccator and weighed. The process of drying, cooling and weighing was repeated at half hour intervals till constant weight was achieved and the difference between two consecutive weights was less than 1 mg. Each time before weighing, the Petri dish was cooled in desiccators. Moisture content of the sample was expressed in g/100 g of sample.

Calculation:

$$\text{Moisture \%} = \frac{\text{W1 (g)} - \text{W2 (g)}}{\text{W (g)}} \times 100$$

Where, W1 = Weight of the petri dish with the sample before drying (g),

W2 = Weight of the petri dish with the sample after drying (g) and

W = Weight of the sample (g)

3.7.2 Estimation of ash (IS:1155 – 1968 reaffirmed 2010)

Total ash was estimated using standard protocol. Five grams of dried sample was weighed in crucible, placed on wire gauge and ignited on flame of burner till sample was charred completely (Plate 3.7a). Charred sample was then transferred to crucible and heated in the muffle furnace (Make: Genaxy, Model: 1187/04) for about 4 hours at 600 °C till white or greyish colour ash was obtained (Plate 3.7b). It was then cooled in a desiccator and weighed after reaching room temperature. The crucible was heated again in muffle furnace for 1 hour to ensure complete ashing. This process of heating, cooling and weighing was repeated till difference between two consecutive weights was less than 1 mg and ash was white or greyish in colour.

Calculation:

$$\% \text{ Total ash} = \frac{(W_2 - W)\ g}{(W_1 - W)\ g} \times 100$$

Where, W – Weight (g) of the empty crucible, W1 – Weight (g) of the crucible + sample, W2 –Weight (g) of the crucible + ash, Weight of sample = (W1 – W) and Weight of the ash = (W2 – W).

(a) (b)

Plate 3.7 Charring of samples (a) and Muffle Furnace (b)

3.7.3 Estimation of protein (AOAC 954.01 – 2010)

The nitrogen content of the sample was assessed by Kjeldahl method using Pelican Kelplus (Model: KES-08-L-RTS) equipment (Plate 3.8). Crude protein was calculated by multiplying with a factor 6.25.

Principle: The digestion mixture acts as catalyst and digests organic nitrogenous compounds in food in presence of sulphuric acid and are converted to ammonium sulphate. Ammonium liberated by making the solution alkaline is distilled into a known volume of standard acid, which is then back titrated. Protein per cent was calculated by multiplying the nitrogen present by the factor 6.25.

Reagents

1. 4 % boric acid solution: 40 g of boric acid was dissolved in some distilled water, and solution was then transferred to a 1000 ml volumetric flask and made up to the mark.
2. 40 % NaOH (w/v): 400 g of NaOH was stirred continuously and dissolved in some distilled water taken in beaker, kept in basin containing cold water. Then it was transferred to 1000 ml volumetric flask and volume was made up to mark.
3. 0.1N HCl: 8.33 ml of fuming HCl was dissolved in 1000 ml of distilled water.
4. Mixed indicator was made by mixing methyl red (0.2 %) and Bromocresol green (0.2 %) in a 1:2 ratio (v/v) respectively.
5. Digestion mixture was prepared by adding 98 g of potassium sulphate and 2 g of copper sulphate.
6. Concentrated sulphuric acid (H_2SO_4)

Procedure:

Digestion: 0.5 g of the samples was weighed into the digestion tubes and one gram of digestion mixture was added to each tube. Ten ml of concentrated sulphuric acid was also added and samples were digested until the contents of the tubes were clear or sea green in colour. Blank was run simultaneously without the sample. All samples were cooled before distillation.

Distillation: The digested sample tube was placed on automatic distillation unit furnished with 40 per cent NaOH and 4 per cent boric acid and distilled water to facilitate operation. In the collecting conical flask 3 – 4 drops of mixed indicator were added where liberated ammonia is trapped. Distillation was done for 9 minutes and the ammonia collected and trapped by the boric acid in between of samples, the unit was rinsed with distilled water for two and half minutes. The boric acid turned from reddish pink to green as it collected the ammonia.

Titration: The green coloured boric acid was titrated against the 0.1N HCL until its colour turned to pink. A blank was run simultaneously. The titre values obtained were incorporated in the equation below to obtain the per cent nitrogen (N) present in the sample which, in turn, was multiplied by the factor 6.25 to obtain the per cent protein.

Calculation:

$$\text{Per cent N} = \frac{(V_a - V_b) \times \text{Normality of HCL} \times 14.00}{\text{Sample weight (g)} \times 1000} \times 100$$

Per cent protein = per cent N × 6.25,

Where, V_a =Titre value of sample, V_b = Titre value of blank

Plate 3.8 Sample digestion using Kjeldahl equipment for protein estimation

3.7.4 Estimation of crude fat (AOAC 922.06 – 2007)

Fat from bran samples, best accepted muffins and buns were estimated as crude ether extract using moisture free sample. The solvent was removed by evaporation and the residue of fat was weighed.

Principle: Fat extraction from sample was done using Soxhlet apparatus (Plate 3.9) where the solvent comes in contact continuously with sample placed into thimbles and fat can be extracted.

Procedure: Two grams of sample was weighed into a thimble and plugged with fat free cotton wool. Thimble was placed in the pre-weighed Soxhlet extraction beakers filled with petroleum ether. The sample was extracted for about 2 hours in fat extraction unit (Socsplus SCS 06 AS, Pelican). After extraction the beakers were retrieved from apparatus when little solvent was left and the beakers were transferred in hot air oven (Make: Ria Instruments) to evaporate the remaining solvent, leaving behind only the crude fat extracted. Beakers were then cooled in desiccator and weighed to estimate the fat content on sample. The fat extracted was expressed as grams per 100 g.

Calculation:

$$\text{Fat content (g/100g)} = \frac{\text{Weight of ether extract (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Where,

Weight of the ether extract =W1 – W; W (g) = Initial weight of the beaker; W1 (g) = Weight of the beaker + ether extract

Plate 3.9 Fat extraction unit

3.7.5 Estimation of crude fibre (AOAC 962.09 – 2007)

Crude fibre of the sample was estimated by using moisture and fat free samples and expressed as g/100 g of the sample

Principle: During the acid and subsequent alkali treatment, oxidative hydrolytic degradation of native cellulose and considerable degradation of lignin occurs. The residues obtained after final filtration is weighed, incinerated, cooled and weighed again. The loss in weight is the crude fibre content.

Reagents

1. Standards H_2SO_4 (1.25 %)
2. Standard NaOH (1.25 %)
3. Alcohol (95 %)

Procedure: Moisture and fat free sample was weighed accurately (1-2 g) and was transferred to fibre bags, which was pre weighed. The bags were inserted in the tubes and then kept in the beaker provided with the equipment (Make:Gerhardt, Model: EV-1). Firstly, the samples were boiled with 200 ml of H_2SO_4 for 30 minutes followed by washing in boiling water to make the sample acid free. Then the samples were boiled in 200 ml NaOH solution for 30 minutes. Again, the samples were then washed with boiling water followed by alcohol wash. The residue was then transferred to a pre- weighed ashing dish, dried for 2 hours at 130 ± 2 ºC, cooled in a desiccator and weighed (W_e). The dry dishes containing the sample were then ignited for 40 minutes at 600 ± 15 ºC. Finally, the samples were cooled in desiccator and then weighed again (W_a).

Calculation:

$$\text{Crude fibre (g/100 g)} = \frac{(W_e - W_a)}{\text{Wt. of sample taken (moisture and fat free)}} \times 100$$

Where,

W_e- pre-weighed ashing dish + sample after drying;

W_a- weight of the dish after ashing (sample + dish)

3.7.6 Estimation of total dietary fibre (AOAC 985.29 – 2010)

Total dietary fibre (TDF) was estimated using standard protocol with slight modification based on enzymatic and gravimetric methods. Moisture and fat free samples were weighed (1 g) accurately and subjected to gelatinization with heat stable α-amylase and then digested with protease and amlyoglucosidase for removing starch and protein content in the samples. This was followed by ethanol precipitation of soluble dietary fibre. Residue was then filtered washed with ethanol and acetone, followed by drying. Half of the samples were then analysed for protein and other half for ash. TDF was weight of residue minus the weight of protein and ash.

Reagents:

1. 95 % and 78 % ethanol
2. Phosphate buffer 0.08 M, pH 6.0.
3. Sodium hydroxide solution, 0.275 N.
4. Hydrochloric acid solution, 0.325 N.
5. Protease solution (50 mg/ml)

Procedure: Blanks were run throughout the procedure. Accurately four 1 g samples were weighed taken in 500 ml conical flasks, to which 50 ml of phosphate buffer and 0.1 ml of α-amylase was added. Beakers were covered with aluminium foil and placed in boiling water bath shaker. Samples were agitated gently at 5 min interval and incubated for 15 min till inter temperature reached 95 °C. Samples were then cool to room temperature and pH 7.5 ± 0.1 was adjusted by adding 10 ml 0.275 N NaOH solution. To this 0.1 ml of protease solution was added, beakers were covered with aluminium foil and incubate at 60 °C with continuous agitation for 30 min. The samples were then cooled and 10 ml 0.325 N HCl solution was added to adjust pH to 4.5 ± 0.2. To this solution 0.1 ml of amyloglucosidase was added. Beakers were cover with aluminium foil, and incubate for 30 min at 60 °C with continuous agitation. Four volumes of 95 per cent EtOH was added and solutions were allowed to set overnight at room temperature. These samples were filtered next day on crucible containing celite bed (pre-weighed) and the residue was washed with three 20 ml portions of 78 per cent EtOH, two 10 ml portions of 95 per cent EtOH, and two 10 ml portions of acetone. The crucible containing residue were dried overnight at 105 °C in hot air oven. The crucibles were cooled in desiccator and weigh to nearest 0.1 mg, and residue weight was determined by subtracting crucible and Celite weights. Out of four set of samples, one sample of set of duplicates and blank was analysed for protein (Kjeldahl nitrogen analyser) using N x 6.25 as conversion factor. And other set of duplicates and blank was used to estimate ash. Samples were incinerated at 525 °C for 5 hours. Crucible and celite weight were subtracted to determine ash.

Calculation:

$$\text{Blank protein residue (BPR)} = \frac{\text{Average weight of blank} \times \text{\% protein}}{100}$$

$$\text{Blank ash residue (BAR)} = \frac{\text{Average weight of blank} \times \text{\% ash}}{100}$$

Corrected blank (CB) = average blank weight – BPR – BAR

$$\text{Sample protein residue (SPR)} = \frac{\text{Average weight of sample} \times \text{\% protein}}{100}$$

$$\text{Sample ash residue (SAR)} = \frac{\text{Average weight of sample} \times \text{\% ash}}{100}$$

Corrected sample residue (CSR) = average sample weight – SPR – SAR – CB

% Total dietary fiber (TDF) = 100 × CSR.

3.7.7 Estimation of calcium (Ca), iron (Fe) and zinc (Zn) using Atomic Absorption Spectrophotometry (AAS) (AOAC 975.03 – 1990)

Moisture free bran samples and products were wet digested using microwave digester to estimate mineral (Fe, Ca, Zn, Na and K).

Wet digestion: Accurately weighed samples (0.5 g) were transferred in microwave digestion tubes carefully. A blank tube was run with each set of samples to detect mineral contribution from nitric acid. To each digestion tube, 10 ml of concentrated nitric acid was added and samples were held up 10 minutes for pre-digestion. The tubes were then closed properly and loaded into the rotor which was placed inside microwave digester. The digestion was started by selecting suitable program in microwave digester (Multiwave Go) instrument (Plate 3.10). The temperature initially rises to 120 °C within 10 minutes and holds the sample at 120 °C for 5 minutes, then the temperature rises to 180 °C and the samples are held at 180 °C for 15 minutes. After this digestion process, samples were cooled to 55 °C. After completion of digestion and cooling the rotor was unloaded and the digestion tubes were removed from rotor. These tubes were immediately placed in fume hood and slowly caps of all digestion tubes were opened to let the brown nitric oxide fumes escape from the tubes. The samples were then transferred into 50 ml standard volumetric flask. The tubes and caps were cleaned with ultrapure water into the standard flask and volume was made up to 50 ml and transferred into storage container. These samples were further analysed in AAS (Plate 3.11) for estimating minerals.

(a) Putting sample in digester tube

(b) Placing tubes on rotor

(c) Setting microwave digester

(d) Microwave digester

Plate 3.10 Microwave digester

Plate 3.11 Atomic Absorption Spectrophotometry (AAS)

Estimation of Iron and Zinc by AAS

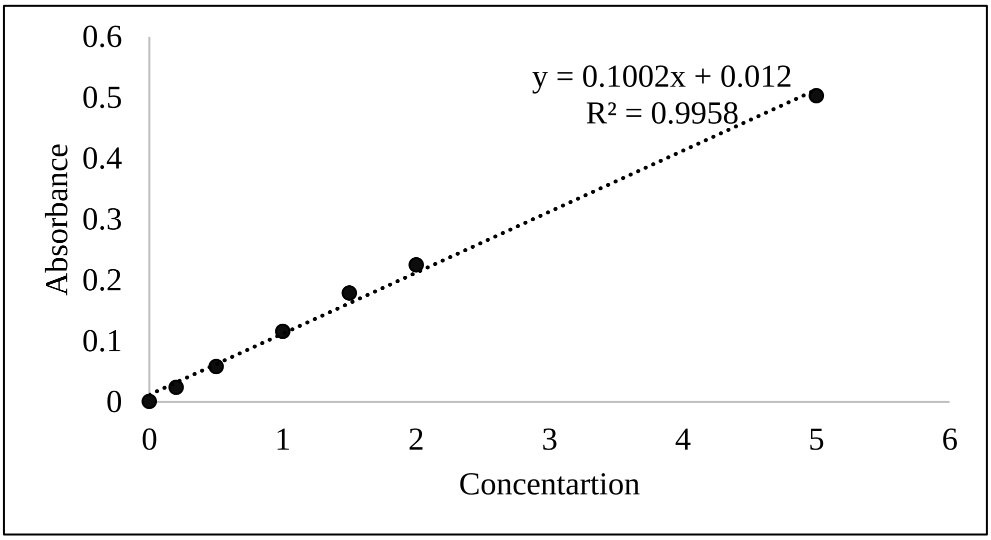
Preparation of Iron Standard: Aliquot of 500 µl from 1000 ppm iron standard was taken in 50 ml volumetric flask and volume was made up to 50 ml with ultrapure water to get 10 ppm stock solution. From this stock solution aliquot of 1.0 ml, 2.5 ml, 5.0 ml, 7.5 ml, 10.0 ml and 25 ml were taken into standard 50 ml volumetric flask and volume was made up to 50 ml with 0.5 M nitric acid to get 0.2 ppm, 0.5 ppm, 1.0 ppm, 1.5 ppm 2.0 ppm and 5.0 ppm standards respectively ($y = 0.1002x + 0.012$; $R^2 = 0.9958$ for brans and $y = 0.0979x + 0.0098$; $R^2 = 0.9979$ for products). Standard curve is depicted in Fig 3.4.

Preparation of Zinc Standard: Aliquot of 250 µl from 1000 ppm zinc standard was taken in 50 ml standard volumetric flask and made up to 50 ml with ultrapure water to get 5 ppm of stock solution. From the stock solution, an aliquot of 1.0 ml, 3.0 ml, 5.0 ml, 7.5 ml and 10.0 ml in to 50 ml standard flask and volume was made up to 50 ml with 0.5 M nitric acid to get 0.1 ppm, 0.3 ppm, 0.5 ppm, 0.75 ppm and 1.0 ppm standards respectively ($y = 0.5821x + 0.0107$, $R^2 = 0.9981$ for brans and $y = 0.5818x + 0.0231$, $R^2 = 0.992$ for products). Standard curve is depicted in Fig 3.5.

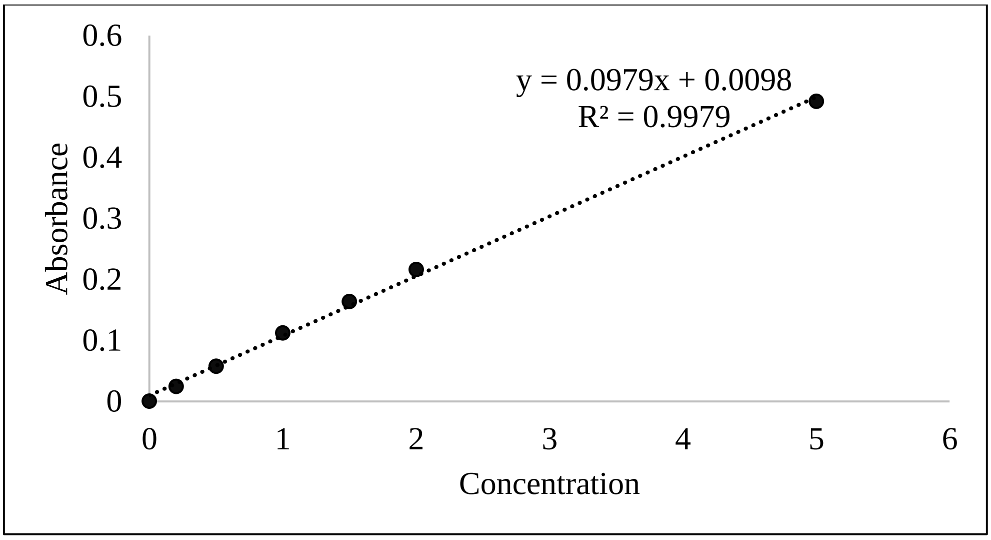
Procedure: The AAS was set up and iron or zinc method was created. Instrument was optimized and standards were fed to obtain standard curve, 0.5 M nitric acid was used as blank. Digested blank and samples were aspirated to measure absorbance which was converted to concentration using the linear equations. Blank value was subtracted from all the samples.

Calculation:

$$\text{Fe or Zn (g/100g)} = \frac{\text{Concentration from standard curve} \times \text{Volume made up} \times \text{Dilution factor} \times 100}{\text{Weight of sample taken (g)} \times 1000}$$

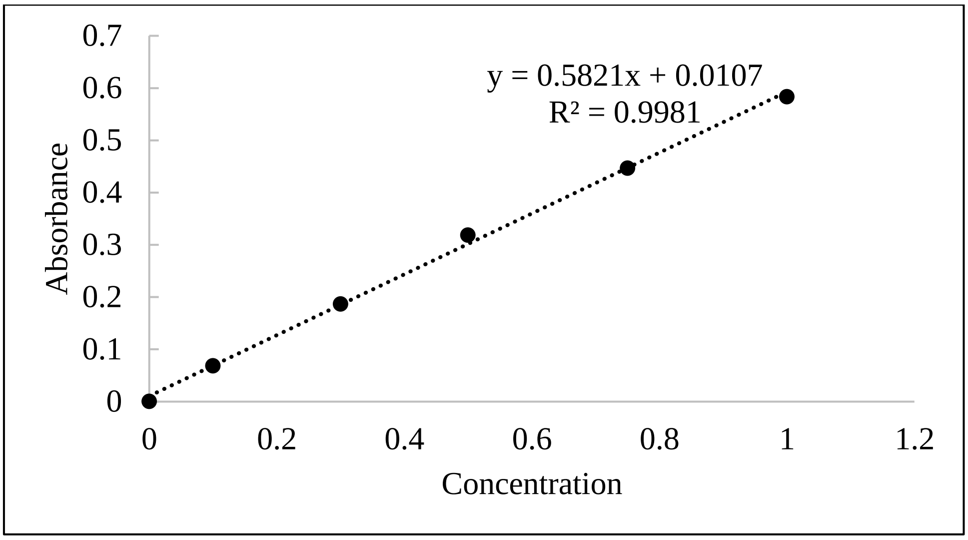


(a)

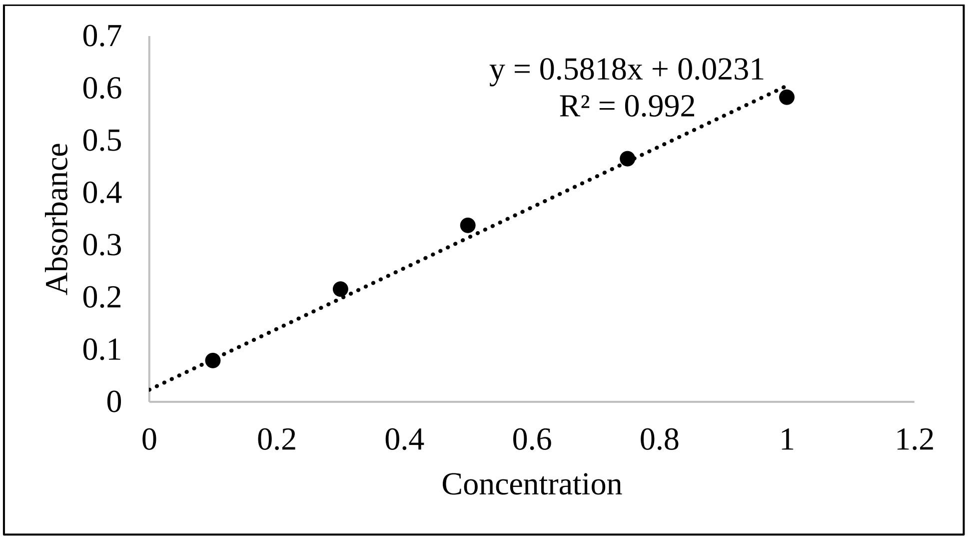


(b)

Fig. 3.4 Standard curve for iron in bran (a) and products (b)



(a)



(b)

Fig. 3.5 Standard curve for zinc in bran (a) and products (b)

Estimation of Calcium (Ca) by AAS (Air: Acetylene flame)

Calcium determination requires addition of Lanthanum to the standards and samples to avoid oxyanion interference. On general occasions 0.1 per cent Lanthanum concentrations are recommended. But with samples having higher calcium content like finger millet 0.4 – 0.5 per cent of Lanthanum concentration is desired.

Preparation of Lanthanum solution (20 %): Accurately weighed 53.47 g of $LaCl_3.7H_2O$ was dissolved in 50 ml of ultrapure water and made up to 100 ml.

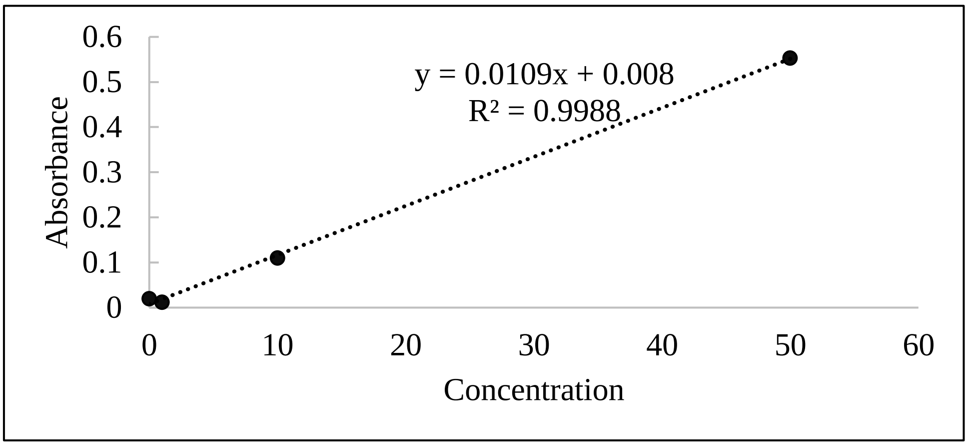
Preparation of calcium standard: Calcium standards were prepared for 1 ppm, 10 ppm and 50 ppm for this aliquot 0.025 ml, 0.25 ml and 1.25 ml were taken respectively from stock solution of 1000 ppm standard calcium and transferred into 25 ml volumetric flask.

To each standard 0.5 ml of 20 per cent lanthanum chloride was added to get final concentration of 0.4 per cent and volume was made up to 25ml with 0.5 M nitric acid ($y = 0.0109x + 0.008$, $R^2 = 0.9988$ for brans and $y = 0.0152x - 0.0039$, $R^2 = 0.9959$ for products). Standard curve is depicted in Fig 3.6.

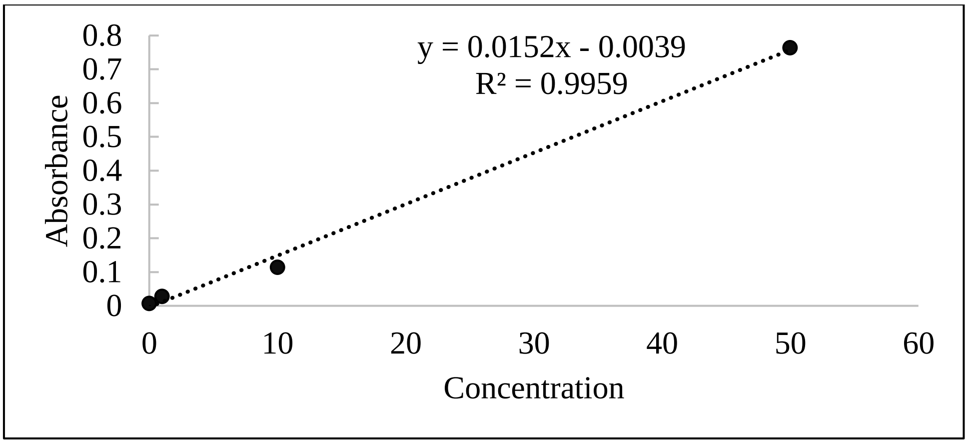
Procedure: The AAS was set up and calcium method was created. Instrument was optimized and standards were fed to obtain standard curve, 0.5 M nitric acid was used as blank. Aliquot (0.5 ml) of Lanthanum chloride (%) was added to 9.5 ml digested sample and these samples were aspirated along with blank to measure absorbance which was converted to concentration using the linear equations. Blank value was subtracted from all the samples.

Calculation:

$$\text{Ca (g/100g)} = \frac{\text{Concentration from standard curve} \times \text{Volume made up} \times \text{Dilution factor} \times 100}{\text{Weight of sample taken (g)} \times 1000}$$



(a)



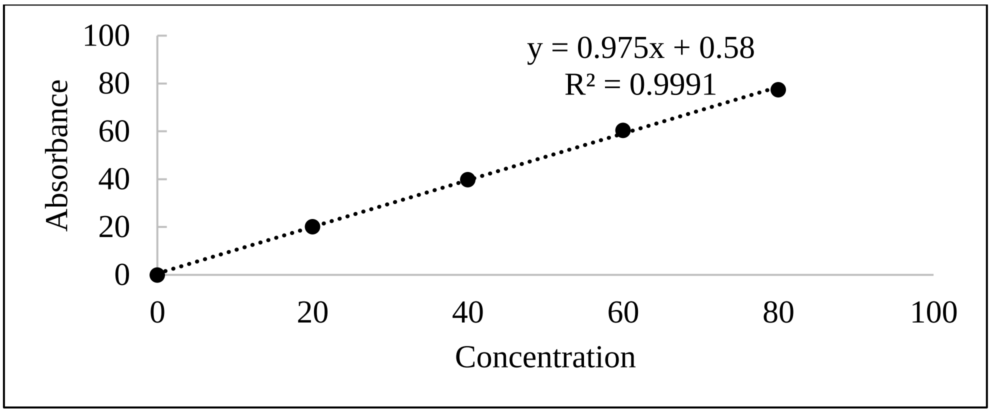
(b)

Fig. 3.6 Standard curve for calcium in bran (a) and products (b)

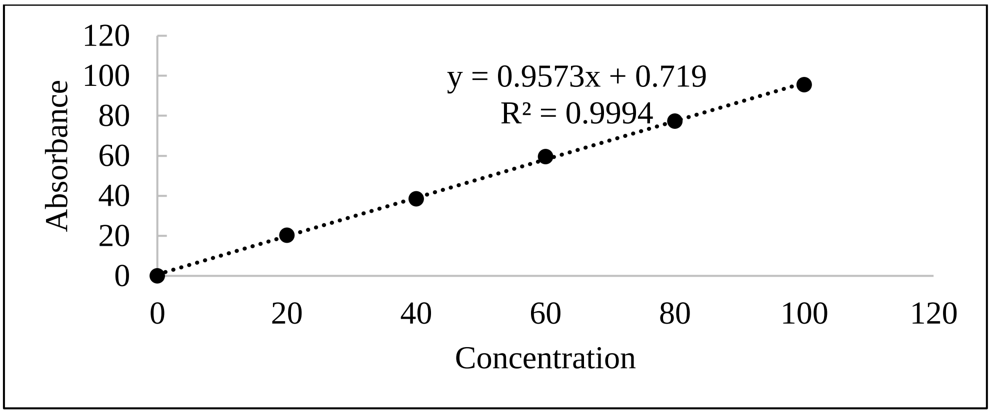
3.7.8 Estimation of Sodium and Potassium using Flame Photometer (AOAC 956.01 – 1990)

Sodium and potassium were estimated using a flame photometer (Make: Elico, Model:CL-378) (Plate 3.12). The samples wet digested using nitric acid (as given under section 3.7.6) were used for estimation.

Preparation of Sodium (Na) and Potassium (K) standards: Stock solution of 1000 ppm of Na and K was prepared using 2.542 g of NaCl and 1.907 g of KCl in ultrapure water (1000ml) respectively. From the stock solution working standards of 20 ppm to 100 ppm was prepared for Na and 20 ppm to 80 ppm was prepared for K. Standard curve was obtained and the samples were read against the standard curve (Fig 3.7 and 3.8). sodium is read at 589 nm and potassium at 766 nm. The line equation was $y = 0.975x + 0.58$; $R^2 = 0.9991$ for sodium in bran samples and $y = 0.9573x + 0.719$, $R^2 = 0.9994$ for products; $y = 1.0383x - 0.5476$; $R^2 = 0.9997$ for potassium in bran samples and $y = 1.0079x - 0.1095$, $R^2 = 0.9998$ in products. Distilled water was used as blank.

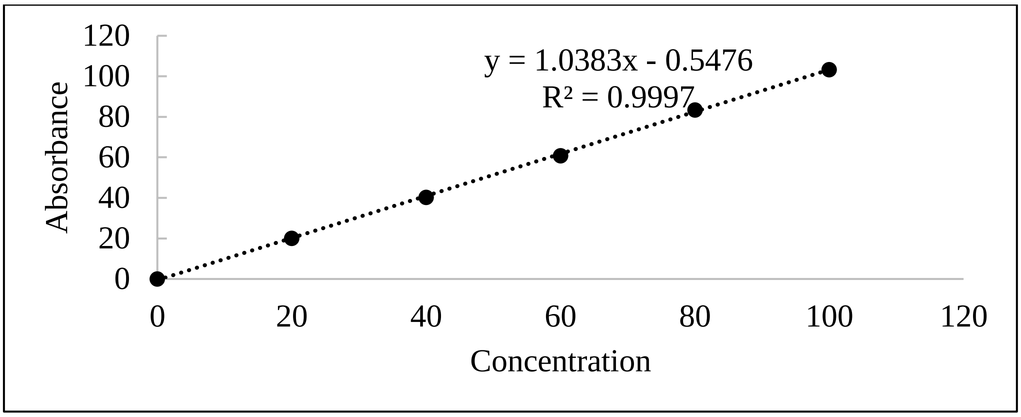


(a)

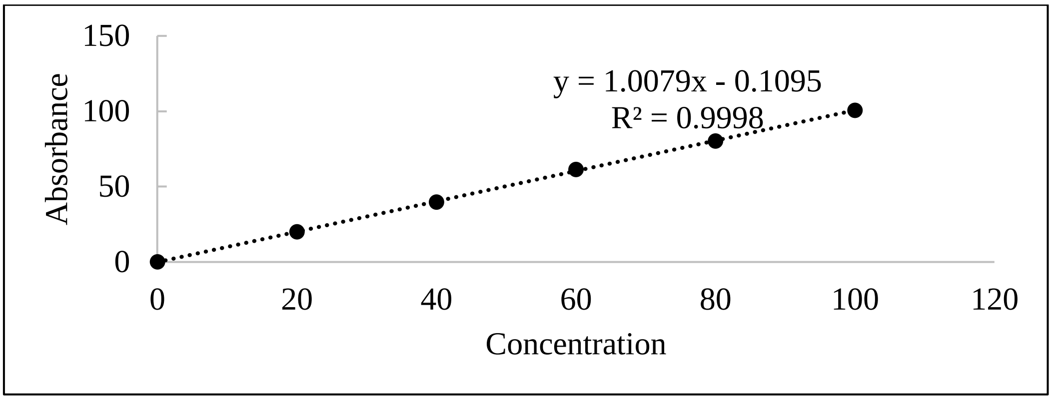


(b)

Fig. 3.7 Standard curve for sodium in bran (a) and products (b)



(a)



(b)

Fig. 3.8 Standard curve for potassium in bran (a) and products (b)

Plate 3.12 Flame photometer

3.7.9 Computation of carbohydrate (AOAC, 2006)

Total Carbohydrate content was calculated 'by difference' method using following formula:

Total Carbohydrate (g/ 100 g) = 100 - [Protein (g) + Fat (g) + Ash (g) + Moisture (%)]

Available carbohydrate was calculated 'by difference' method using following formula:

Available Carbohydrate (g/ 100 g) = 100 - [Protein (g) + Fat (g) + Dietary Fibre (g) + Ash (g) + Moisture (%)]

3.7.10 Computation of energy (AOAC, 1980)

Energy was computed as follows for all the samples.

Energy (Kcal) = [Protein (g) x 4] + [Carbohydrate (g) × 4] + [Fat (g) × 9]

3.7.11 Estimation of antioxidant using DPPH (Blois, 1958)

Methanolic extract of sample as quoted in 3.4.1 was used for estimation of antioxidants using DPPH method. Standard procedure was followed with slight modification (Blois 1958). Aliquot of the sample (0.1ml or 0.2ml) was taken to which 3ml of 1 Mm methanolic solution of DPPH was added. The mixture was incubated at 37°C for 20 min in dark and absorbance was measured at 517 nm by using spectrophotometer (Make: Shimadzu, Double Beam UV-VIS SPM, Model: UV-1800). The activity was expressed as percentage DPPH scavenging relative to control. One millilitre of methanol was treated same as sample and used as control. Standard curve was obtained using Trolox (Conc. 1 µg-5 µg) and treated in same way as samples (Fig 3.9).

Calculation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

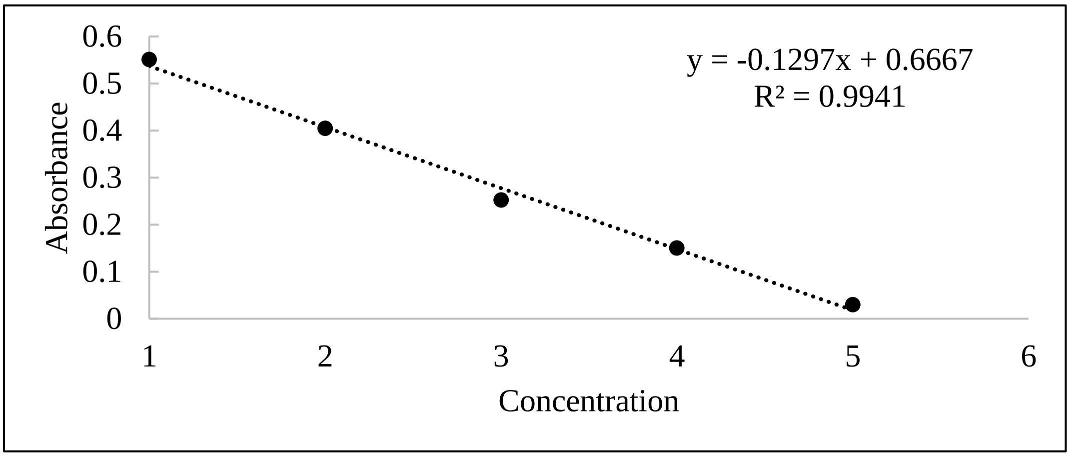


Fig. 3.9 Standard curve for DPPH in bran and products

3.7.12 Estimation of TBARS (Zeb and Ullah, 2016)

Standard protocol as described by Zeb and Ullah (2016) with slight modification.

Reagents:

1. Thiobarbituric acid (TBA) 4 mM: 57.66 mg TBA was dissolved in 100 ml glacial acetic acid.
2. Malondialdehyde (MDA) stock solution 1 mM: 31.35 mg of MDA was dissolved in 100 ml glacial acetic acid. Working standard with 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM were prepared from stock solution to obtain standard curve (Fig 3.10).

Extraction of sample: Sample used to estimate TBARS were extracted using glacial acetic acid. To accurately weighed 2 g finely ground samples 10 ml of 100 per cent glacial acetic acid was added and the mixture was shaken for 1 hour continuously, followed by filtration with Whatman No 1 filter paper. The extract obtained was further diluted to 5 ml using glacial acetic acid.

Procedure: The extract of each sample (2 ml) was mixed with 2 ml TBA reagent. the mixture was incubated in boiling water bath (95 °C) for 60 minutes. Working standards were treated the same as sample and read at 532 nm to obtain standard curve. The TBARS was calculated using the formula as μM/g of the sample:

$$\text{TBARS } (\mu\text{M/g}) = \frac{\text{Ac} \times \text{V}}{\text{W}} \times 100$$

Where Ac is the amount determined from the calibration curve and W is the weight of the sample taken while V is volume in ml of the total extract prepared.

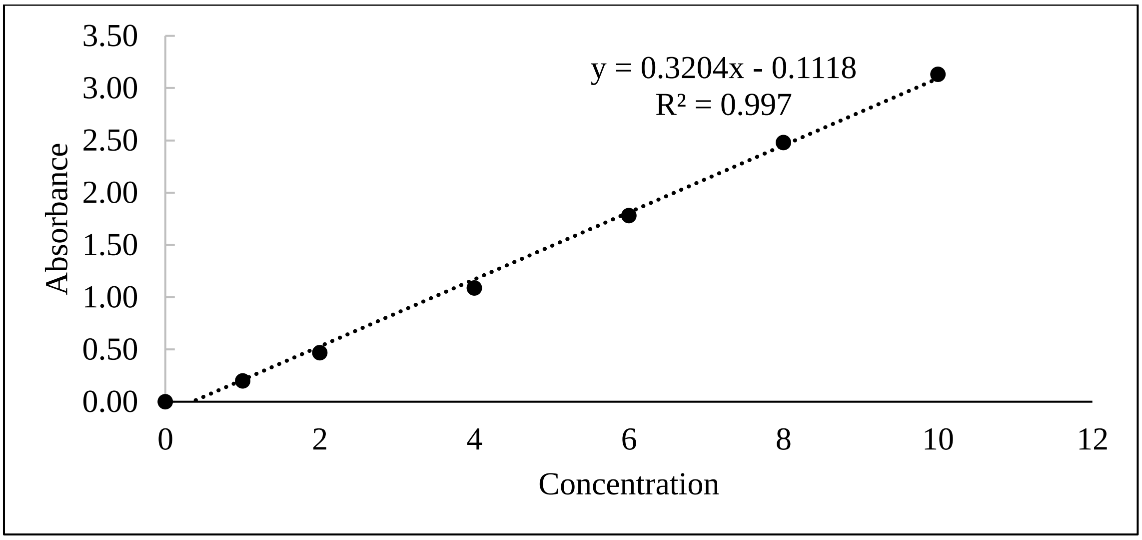


Fig. 3.10 Standard curve for TBARS in bran and products

3.13 Estimation of FRAP (Ferric Reducing Antioxidant Power) (Benzie and Strain, 1999)

FRAP protocol was followed with slight modification as described by Benzie and Strain (1999). Methanolic extract of sample as quoted in 3.4.1 was used for antioxidant estimation with FRAP.

Principle: FRAP assay uses antioxidants in sample as reductants which reduced Fe3+-TPTZ complex, reaction where colourless ferric form is reduced to blue coloured complex ferrous form which is read at 593 nm. The absorption readings are related to the reducing power of electron donating antioxidant present in the compound.

Reagents:

1. 300mM Acetate buffer (3.1g sodium acetate trihydrate +16 ml glacial acetic acid, made up to 1000ml with distilled water)
2. 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) was prepared in 40 mM HCl.
3. 20 mM $FeCl_3$

Procedure: FRAP reagent was prepared by mixing Acetate buffer, TPTZ and $FeCl_3$ in 10:1:1 proportion. Standard curve was obtained using Trolox (0.05 to 0.3 mM). Samples/ standards were mixed with 3 ml distilled water and 1.8 ml FRAP reagent. This mixture was incubated for 4 minutes at 37 °C and samples were read at 593nm. FRAP reagent (1.8ml) and distilled water (3ml) was used as blank. Concentration was obtained from the standard curve ($y = 0.8493x + 0.0079$, $R^2 = 0.986$ for brans and $y = 0.3057x + 0.0117$ $R^2 = 0.9689$ for products) (Fig 3.11).

Calculation:

$$\text{FRAP } (\mu M/g) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 1000\ (\mu mol/g)$$

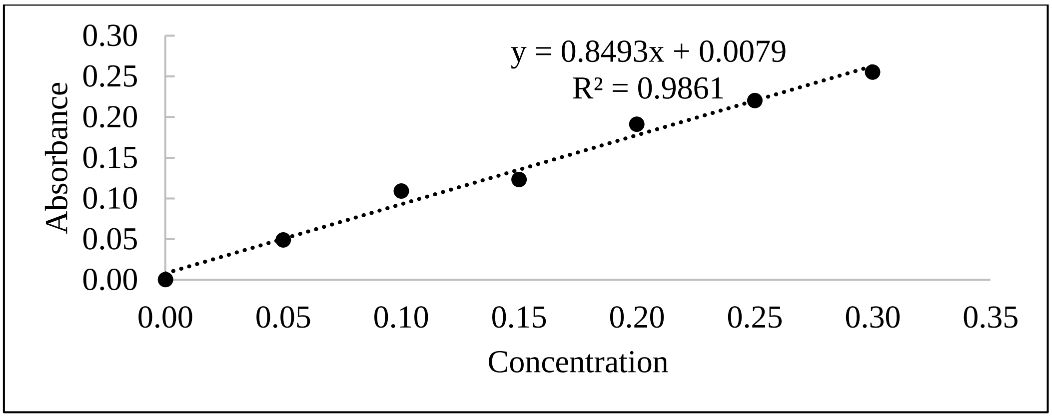


Fig. 3.11 Standard curve for FRAP in bran and products

3.8 Physical and functional properties minor millet brans, bran blended flours and designer products

Physicochemical properties of brans and flours used for muffins and buns preparation were analyzed. According to the sample suitability different properties viz., water absorption and water solubility index, oil absorption capacity and foaming capacity were measured. Weight and height of the products (muffins and buns) were measured and baking loss rate was calculated.

3.8.1 Water absorption capacity (WAC) and water solubility index (WSI) (Sharma *et al.* 2004 and Sairam *et al.* 2011)

Best stabilized bran from each grain were further analyzed for water absorption and solubility using standard protocol with slight modification. Briefly 1 g of sample was weighed accurately to which 30 ml of distilled water was added and mixed properly. This mixture was allowed to stand for 30 minutes at room temperature, then it was centrifuged for 3000 rpm for 30 minutes. The amount of water retained by the sample was measured and results were expressed as Water absorption capacity g/100g. For water solubility, the supernatant was carefully collected in pre weighed Petri dish (W1) dried in hot air oven at 100 °C till dry and weight was recorded (W2). Water solubility was calculated and expressed as per cent.

$$\text{Water absorption capacity (g /100 g)} = \frac{\text{Water absorbed by sample}}{\text{Initial weight of sample}} \times 100$$

$$\text{Water solubility index (\%)} = \frac{\text{W2-W1}}{\text{Initial weight of sample}} \times 100$$

3.8.2 Oil absorption capacity (OAC) (Sharma *et al.* 2004 and Sairam *et al.* 2011)

Oil absorption capacity of best stabilized bran samples from each grain was further analyzed using standard protocol with slight modification. Accurately 1 g of sample was weighed to which 10 ml refined oil (V1) was added, this mixture was mixed properly using vortex mixture and allowed to stand for 30 minutes at room temperature, then it was centrifuged for 3000 rpm for 30 minutes. The supernatant was carefully decanted in graduated cylinder and volume was recorded (V2). Difference between initial and final volume were calculated and oil absorption capacity was determined.

$$\text{Oil absorption capacity (g /100 g)} = \frac{\text{V1-V2}}{\text{Initial weight of sample}} \times 100$$

3.8.3 Foaming capacity (FC) (Chandra *et al.* 2015)

Foaming capacity was determined by using protocol described by Chandra *et al.* (2015) with slight modifications. Accurately 1 g of sample was measured and transferred in graduated cylinder, to which 50 ml distilled water (V1) was added. This mixture was shaken to mix well and then homogenised for 5 minutes to foam. The volume of foam at 30 seconds was noted (V2) and foaming capacity was calculated using following formula.

$$\text{Foaming capacity (\%)} = \frac{\text{V2-V1}}{\text{V1}} \times 100$$

Where, V1 = volume before whipping, V2 is volume after whipping.

3.8.4 Weight and height

Weight of muffins and buns were recorded using a precision weighing balance before and after baking. Height of muffin and buns before and after baking was measured using calipers to nearest millimeter.

3.8.5 Baking loss rate (Heo *et al.* 2019)

Baking loss rate (BLR) was calculated for both buns and muffins as per standard protocol described by Heo *et al.* (2019). Average reading of 5 samples were considered and calculation was done using the following formula:

$$\text{Baking rate loss (\%)} = \frac{\text{Weight of batter – weight of baked product}}{\text{Weight of batter}} \times 100$$

3.9 Shelf life of best accepted minor millet bran incorporated designer products

Buns and muffins had short span of shelf life as no preservatives were added during product standardization. Both best accepted buns and muffins selected for feeding studies along with control samples were observed only for 7 days. Parameters like moisture, water activity, microbial plate count and sensory evaluation were carried out during storage period using standard protocols.

3.9.1 Estimation of moisture (IS:1155 – 1968 reaffirmed 2010)

Moisture estimation was done at 0, 3rd and 7th day using standard protocol (IS:1155 – 1968 reaffirmed 2010) mentioned previously under section 3.7.1 in this chapter. Briefly both muffin and bun samples were dried at 105 ± 2 °C in a hot air oven for 4 hours, cooled and weights were recorded until a constant weight was recorded.

3.9.2 Water activity

Amount of water and its distribution in the product affects its shelf life. Thus, it is important factor in predicting the shelf life. Water activity was analyzed for samples at 0, 3rd and 7th day using standard protocol as described by (Clavero *et al.* 2000). Lab Touch advanced water activity meter (Make: Novosina) was used to conduct the experiment (Plate 3.13). The equipment was calibrated using concentrated salt standards of known relative humidity and 20 °C temperature was maintained. The samples were accurately weighed (2 g) in small plastic cups and placed in the equipment after the calibration was complete. The values were recorded and all samples were analysed in triplicate.

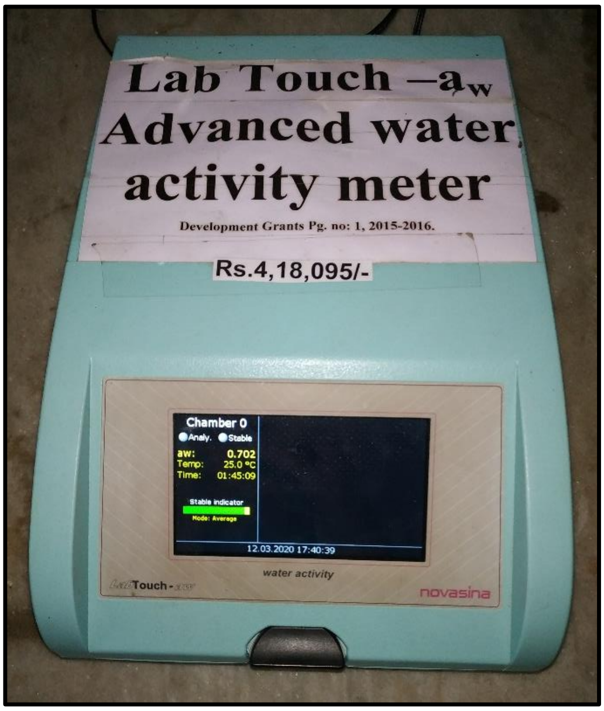
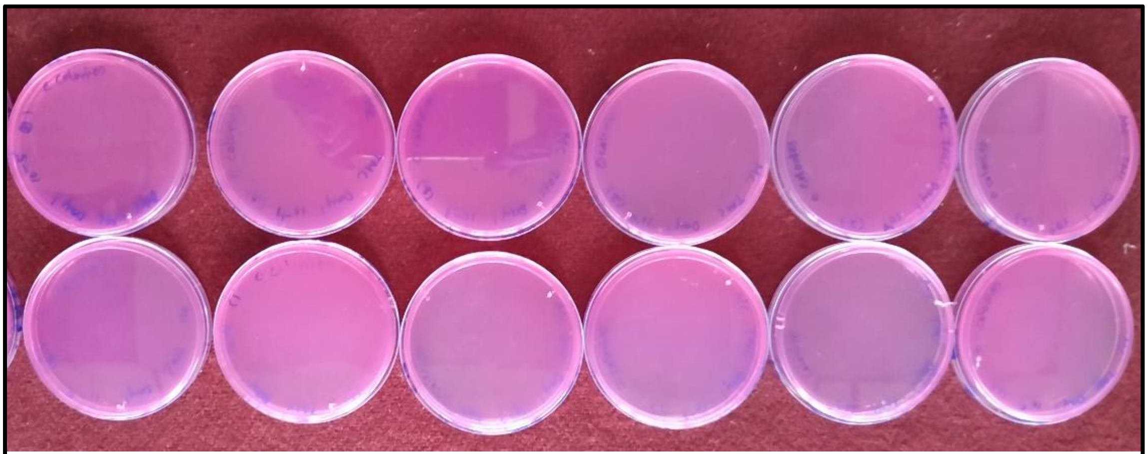


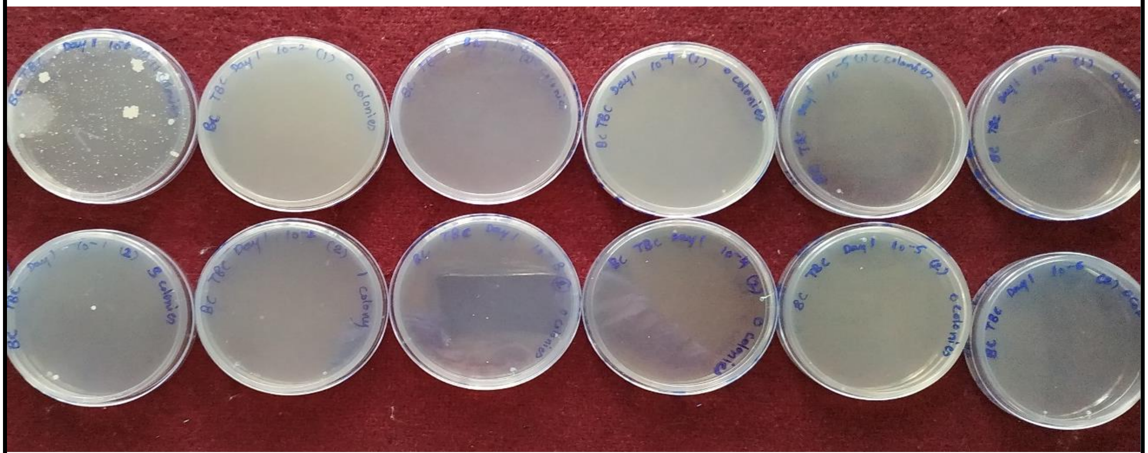
Plate 3.13 Water activity meter

3.9.3 Microbial plate count

Microbial spoilage is one of the concerns, limiting shelf life of bakery products like breads, muffins, cakes and buns (Smith *et al.* 2004). Pour plate method was followed and standard plate count was noted (Goswami *et al.* 2015). Spoilage of buns and muffins are mainly caused by fungus / moulds thus, using standard protocol pour plating was carried out on Martins Rose Bengal Agar (MRBA) for total fungal count and Nutrient Agar (NA) for total bacterial count. Each sample was weighed accurately (1 g) and mixed in 10 ml of saline water blank. Further serial dilution was continued up to 10^{-6} by pipetting 1 ml which was added to 9 ml saline water blank. All dilutions were plated in sterile petri plates, and the media was poured in the plates rotated clock and anti-clock wise (Plate 3.14). These plates were then incubated in inverted position for 48 hrs (bacteria) and 72 hours (fungi) at 32 ºC after which the colonies were counted using colony counter (Make: Equitron).



(a) Petri plates plated for total fungal count using Martins Rose Bengal Agar (MRBA)



(b) Petri plates plated for total bacterial count using Nutrient Agar (NA)

Plate 3.14 Microbial analysis of products

3.9.4 Sensory evaluation during shelf life (Peryam and Pilgrim, 1957)

Sensory evaluation of the products was conducted at 0, 3rd and 7th day using nine-point hedonic scale by semi-trained panel (n=21).

3.10 Therapeutic potential of best accepted minor millet bran incorporated designer products

To analyze therapeutic value of products glycemic index and glycemic load was assessed. The products were supplemented for 45 days to healthy adolescent girls and pre- and post-blood samples were taken to see the difference due to supplementation. Approval of institutional ethical committee was obtained before initiation of the glycemic index and feeding study. Bulk production of buns and muffins for supplementation study was done at MPIC, PJTSAU (Plate 3.15 and 3.16).

3.10.1 Assessment of glycemic index (GI) and glycemic load (GL)

3.10.1.1 Subject selection for GI and GL

Ten normal healthy individuals were recruited for the study with their consent (Appendix B) from a pool of 20 subjects. Oral Glucose Tolerance Test (OGTT) was conducted to screen the subjects for any abnormalities in glucose tolerance. For OGTT before test begins fasting glucose level was checked, then a glass of water containing 75g of glucose (dextrose monohydrate powder) was given and blood samples were checked with finger prick test (AccuSure simple, Model No. TD:4183, Manufactured by Taidoc Technology Corporation, Taiwan) after 15, 30, 60, 90 and 120 minutes. Subjects with abnormal values for 2 hours between 140 - 200 mg/dL were eliminated from the study. Along with blood samples, urine samples were also collected at fasting and 120 minutes after consumption of glucose water and tested to see if glucose is found. If a person has diabetes presence of glucose in urine is noted, which otherwise is absent in normal subjects. Thus, subjects who had glucose levels detected in urine sample were also eliminated from the study. Subjects were excluded if they were taking any medication, having any gastrointestinal disorder, intolerant or allergic to any foods. Subjects were also excluded if they felt any discomfort during the study.

(a) Kneading dough

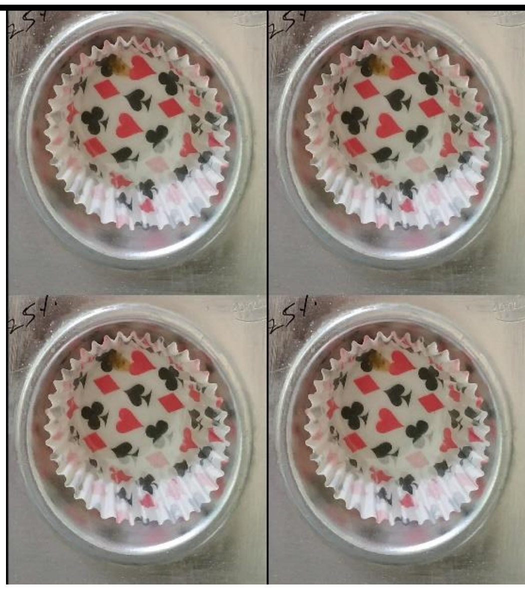
(b) Dough after proofing for 1 hour

(c) Preparing round balls from dough

(d) proofing for 15 mins before baking

(e) Baked buns

Plate 3.15 Production of buns



(a) Preparation of muffins batter (b) Greasing muffins cups

(c) Filling batter in muffins tray

(d) Baked muffins

Plate 3.16 Production of muffins

3.10.1.2 Glycemic index (GI) (Aston *et al.* 2008)

Selected subjects were served 25 g of glucose as reference food and it was repeated for three consecutive days, after a washout period of 4 days test food was given to same subjects. Portion size of test food *i.e.*, kodo bran muffin and foxtail bran bun, was calculated to obtain same amount of available carbohydrate (25 g) as reference food. The amount of available carbohydrate was fixed to 25 g due to large portion size of the test food if 50 g available carbohydrate needs to be given. Before giving reference or test food overnight fasting of 10 hours was maintained. Subjects were also instructed to avoid consumption of heavy meals or excessive exercise on previous day before the testing session. Subjects were asked to consume the given serving of reference/test food within 10-15 minutes. Finger prick test was done using glucometer (AccuSure simple, Model No. TD:4183, Manufactured by Taidoc Technology Corporation, Taiwan) (Plate 3.17). Readings were taken at fasting, 15, 30, 60, 90 and 120 minutes after the consumption of test/reference food. Experiment was conducted thrice for minimizing errors. Incremental area under curve (iAUC) was calculated using trapezoidal rule and GI was calculated with the following formula:

$$\text{GI} = \frac{\text{iAUC for test food}}{\text{iAUC for reference food}} \times 100$$

3.10.1.3 Glycemic load (GL)

Glycemic load was calculated using following formula

$$\text{Glycemic load} = \frac{\text{Dietary carbohydrate content of serving measured in grams}}{100} \times \text{GI}$$

3.10.2 Subject selection for supplementation study (45 days)

Thirty-one normal healthy adolescent girls (BSc 1st year) residing at Professor Jayashankar Telanagana State Agricultural University hostel were selected after conducting meeting for 70 students to advertise regarding participation in the study. In this meeting a questionnaire was filled by potential participants, to know if they suffered from illness in recent past, have any disease or food allergy. Subjects were eliminated if they were having high blood pressure, diabetes, high cholesterol, are on any medication, having any gastrointestinal disorder, intolerant or allergic to any foods. They were also excluded if any discomfort was felt during the study. Written informed consent form

(Appendix C) was obtained after giving complete details of the study in the meeting conducted for selected participants. They were also informed that their participation is voluntary and they can withdraw from the study at any given point. The questionnaire (modified using FAO, 2018) to record the food habits and their general information is presented in the Appendix D. Approval of institutional ethical committee was obtained before initiation of the feeding study and the certificate is presented in Appendix E. Weekly meetings were conducted to take feedback form participants to understand if they faced any discomfort during the study (Plate 3.18).

Study design: Single Arm Study.

The present study was conducted as pre – post design/single arm study, as the objective of study was to collect preliminary data regarding the developed products potential therapeutic benefits. In this study participants enrolled in both groups received either foxtail bran bun or kodo bran muffin. The blood samples were collected, analyzed before and after the feeding.

Plate 3.17 Glucometer

Plate 3.18 Weekly meeting for subjects

Supplementation

Preliminary test was conducted to collect their baseline data regarding glucose, lipid and blood pressure. Baseline data was collected regarding foods consumed and snacking habits using a modified questionnaire (FAO, 2018). Selected participants were randomly divided in two groups (n=15: bun group and n=16: muffin group) which either received one serving of experimental products viz., one foxtail bran bun (70 g) or 2 kodo bran muffins (30 g each) for 45 days continuously. Individually all subjects were provided with either buns or muffins daily depending on their group and were asked to consume the products as snacks or breakfast every day (Plate 3.19).

Blood sample collection

Blood samples were collected by trained lab technician working at Primary Health Center, PJTSAU, Hyderabad. Subjects were taken to Primary Health Center and blood samples were drawn at the initial stage and after supplementation of 45 days (Plate 3.20).

(a) (b)

Plate 3.19 Providing muffins (a) and buns (b) to subjects

Plate 3.20 Drawing of blood samples by lab technician at Health Centre, PJTSAU

3.10.3 Glucose levels

Glucose levels were estimated using standard protocol (GOD-POD method/ end point) at Primary Health Center, Professor Jayashankar Telangana State Agricultural University.

3.10.4 Lipid levels

Total cholesterol (TC), high density lipoprotein (HDL), Low density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides (TG), non-HDL cholesterol, total cholesterol/HDL ratio, triglyceride/HDL ratio, LDL/HDL ratio and LDL/VLDL ratio were estimated at Primary Health Center, Professor Jayashankar Telangana State

Agricultural University using a standard protocol. TC, HDL levels were assessed using standard CHOD/POD end point method, and TG was estimated using GPO/POD Lipase/Glycerol kinase method (Penumarthy *et al.* 2013). LDL and VLDL-C were calculated using equation (Friedewald *et al.* 1972). Non-HDL cholesterol was calculated using the formula: Non-HDL (mg/dl) = TC minus HDL (Virani, 2011).

3.10.5 Protein and albumin content

Total protein and albumin content were also estimated in Primary Health Center, Professor Jayashankar Telangana State Agricultural University using a standard protocol viz., biuret method (Lubran, 1978) and bromocresol purple (BCP) method (Pinnell and Northam, 1978) respectively.

3.10.6 Stress profile

Three parameters were estimated under stress profile *i.e.,* lipid peroxidation, glutathione reductase and catalase activity at Nutrition laboratory, ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad.

3.10.6.1 Lipid peroxidation (Ohkawa *et al.* 1979)

Lipid peroxidation was measured using standard protocol with slight modifications as described by Ohkawa *et al.* (1979). This complex process of lipid peroxidation leads to damage to cell membrane and cell death as it oxidizes polyunsaturated fatty acids of cell membrane.

Principle: MDA (Malonyl dialdehyde) – a secondary lipid peroxidation product, reacts with 2-Thio barbituric acid forming pink coloured trimethyne coloured substance extractable in butanol. It is measured by most of aldehydes that react with TBA derived from peroxidises and unsaturated fatty acids during test produce.

Reagents:

1. SDS (8 % w/v) solution was prepared by dissolving 8 g Sodium Dodecyl Sulphate (1-949540521, MERCK) in 100ml of distilled water and stored in 100 ml reagent bottle.
2. Acetic acid (20 % v/v) was prepared by adding 20 ml Acetic acid (17807, MERCK) to 70 ml distilled water and volume was made up to 100 ml in volumetric flask. Later the solution was stored in 100 ml reagent bottle.
3. TBA (Thiobarbituric acid) was prepared by measuring 0.60 g TBA (RM1594, Himedia) in 100 ml distilled water and stored in 100 ml reagent bottle.

4. Butanol and pyridine (15:1) solution was prepared by mixing 15 ml of butanol (1.01989.0500**,** MERCK) and 1ml pyridine (1.09728.1000**,** MERCK) and stored.

Procedure: In a test tube 100 µl of distilled water was taken to which 50 µl of SDS was added followed by addition of 100 µl of serum. This was incubated at room temperature for 10 minutes. Later 375 µl of 20 per cent acetic acid and 375 µl of TBA was added and placed on boiling water bath for 60 minutes. After this 2.5 ml of distilled water was added to each tube followed by addition of 1.25 ml of 15:1 butanol pyridine solution. The content was then transferred in centrifuge tube and centrifuged at 3000 rpm for 10 minutes (Model: 5804, Make: Eppendorf). The supernatant was collected in 1 ml cuvette and OD was read at 532 nm in spectrophotometer (UV-61Pcs, Metsar). Blank was prepared in same way without addition of serum.

Calculation:

$$\text{Nano moles of Malonyl di aldehyde (MDA)} = \frac{\text{Total volume in test tube } (4.75) \times \text{OD of sample}}{0.156}$$

3.10.6.2 Glutathione reductase

Glutathione reductase was estimated using standard protocol as described by Cohen *et al.* (1970).

Principle: Reductions of oxidised glutathione is catalysed by glutathione reductase in an irreversible reaction producing reduced glutathione and consume NADPH or NADH. This enzyme activity is estimated by measuring rate of decrease in OD by oxidation of NADPH.

Reagents:

1. PBS buffer (0.1M): This was prepared by adding 10 g sodium chloride (RM8530, HI MEDIA), 0.25 g Potassium Chloride (13305, Fisher scientific), 1.44 g Disodium Hydrogen Phosphate (S0246, Rankem) and 0.25 g Potassium Di hydrogen Ortho Phosphate (RM249, Hi media) in 500 ml distilled water.
2. Glutathione Reductase was prepared by dissolving 15 mg Glutathione Reductase (TC134-5G- HIMEDIA) in 5ml distilled water (Freshly prepared)
3. FAD was prepared by dissolving 1mg FAD (RM538-250MG, HI MEDIA) in 5ml of distilled water (Freshly prepared)

4. EDTA was prepared by measuring 3.2 mg EDTA (E0122- Rankem) in 100 ml of distilled water.
5. NADPH was prepared by measuring 16.6 mg of NADPH (RM576-100MG-HIMEDIA) in 10 ml of freshly prepared 1 per cent sodium bicarbonate (27555, Fisher Scientific) solution.

Procedure: In a micro plate 200 µl of 0.1M PBS buffer was added, to which 10 µl of serum was added, blank was kept without addition of serum. Further 10 µl glutathione reductase solution was added followed by addition of 10 µl FAD solution and 10 µl of EDTA solution. Then the plate was incubated for 15 minutes at room temperature. After incubation 10 µl of NADPH solution was added to each well. Further OD was read at 340 nm in microplate ELISA (Model 512101, Make: Venchal Scientific) reader (Plate 3.21).

Calculation

$$\text{Units/ml} \quad = \quad \text{OD of sample} \times 3781$$

Note: 3781 is extinction coefficient

3.10.6.3 RBC catalase (Bergmeyer *et al.* 1986)

Standard protocol as described by Bergmeyer *et al.* (1986) was followed to estimate catalase activity in RBC.

Principle: Hydrogen peroxide gets converted to water and hydrogen in the presence of catalase. Thus, the catalase activity is given by amount of hydrogen peroxide micro molecules consumed per minute per microgram of sample.

Reagents:

1. PBS (0.1M)
2. EDTA (1mM) was prepared by dissolving 40.447 mg of EDTA (E0122, Rankem) in 100ml of distilled water in a volumetric flask
3. H_2O_2 solution was prepared by mixing 3.2 ml H_2O_2 (15465, QUALIGENS) in 100 ml of 0.1 M PBS and stored in 100ml reagent bottle.

Procedure: In a test tube 1 ml of 0.1 M PBS was added, to which 1 ml EDTA was added followed by addition of 50 µl of test sample (RBC) and incubation at room temperature for 10 minutes. Blank was maintained without the test sample. To this 950 µl of H_2O_2 was added and further the samples were diluted to 15 ml. Dilution factor was calculated. Samples were read at 240 nm for rate of change for absorbance per minute.

Calculation:

$$\text{Micro moles of } H_2O_2 \text{ consumed/Min/mg} = \frac{\text{OD of sample} \times \text{DF} \times 1000}{43.6 \times 15}$$

Note: 43.6, 15 are extinction coefficients.

(a) (b)

Plate 3.21 Pipetting samples in micro plate (a) and reading in ELISA reader (b)

3.10.7 Feedback regarding the products from respondents during 45 days of consumption

All the subjects were asked to rate the products using sensory evaluation score card at different stages during feeding study viz., initial day, 15th, 30th and 45th day. Feedback was taken from the subjects to know their preference regarding purchase of such bran blended buns/ muffins from market shelf by asking them to fill a feedback form (Appendix F)

3.11 Statistical analysis

All the experiments were conducted at least in triplicates until otherwise specified and results were analyzed using Microsoft excel and Stat Graphics software (version 18). Multifactorial CRD was used for selecting best stabilization treatments and shelf-life studies of developed designer products. Two factor ANOVA design was used to analyze baking loss rate to understand the effect on each bran and different concentrations. One-way Analysis of variance (ANOVA) was performed for functional properties, nutritional, antioxidant and phytonutrient composition. Paired t-test was used for analyzing the results of grain dimensions and supplementation studies.

Chapter IV

RESULTS AND DISCUSSION

The present work titled "Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential" was conducted in Department of Foods and Nutrition, Post Graduate and Research Centre, Millet Processing and Incubation Centre, Central Instrumentation Cell, Primary Health Centre and Quality Control Laboratory, Professor Jayashankar Telangana State Agricultural University. The study was designed to evaluate minor millet brans viz., Kodo millet (*Paspalum scrobiculatum*), Proso millet *(Panicum miliaceum)*, Barnyard millet *(Echinochloa esculenta)* and Foxtail millet (*Setaria italica)* for their nutritional parameters and utilize it for development of value-add buns and muffins. This chapter presents and discusses results of the study under following sub headings.

4.1 Bran extraction

4.2 Grain dimensions

4.3 Stabilization of brans

4.3.1 Effect of treatment, storage and grain on bran moisture

4.3.2 Effect of treatment, storage and grain on bran FFA

4.4 Functional properties of minor millet brans

4.5 Nutrient profile of minor millet brans

4.5.1 Proximate composition

4.5.2 Mineral content

4.6 Antioxidant capacity and phytonutrient content of minor millet brans

4.6.1 DPPH radical scavenging activity

4.6.2 Ferric Reducing Antioxidant Power (FRAP)

4.6.3 TBARS (Thiobarbituric Acid Reactive Substances) assay

4.6.4 Total phenols (TP) and flavonoids

4.6.5 Phytic Acid (PA)

4.7 Molar ratio of phytate and minerals of minor millet brans

4.8 Physical and functional properties minor millet brans blended flours and their products

4.8.1 Flour blended with minor millet brans

4.8.1.1 Water absorption capacity (WAC) and Water solubility index (WSI)

4.8.1.2 Oil absorption capacity (OAC)

4.8.1.3 Foaming capacity (FC)

4.8.2 Minor millet bran incorporated products

4.8.2.1 Physical characteristics of muffins

4.8.2.2 Physical characteristics of buns

4.8.3 Sensory evaluation of designer muffins and buns

4.9 Nutrient profile of best accepted minor millet bran incorporated designer muffins and buns

4.9.1 Proximate composition

4.9.2 Mineral content

4.10 Antioxidant capacity and phytonutrient content of best accepted minor millet bran incorporated designer muffins and buns

4.10.1 DPPH radical scavenging activity

4.10.2 Ferric Reducing Antioxidant Power (FRAP)

4.10.3 TBARS (Thiobarbituric Acid Reactive Substances) assay

4.10.4 Total phenols (TP)

4.10.5 Phytic acid (PA)

4.11 Shelf life of best accepted minor millet bran incorporated designer muffins and buns

4.11.1 Moisture content

4.11.2 Water activity

4.11.3 Microbial load

4.11.4 Sensory evaluation

4.12 Therapeutic potential of best accepted minor millet bran incorporated designer muffins and buns

4.12.1 Glycemic index (GI) and Glycemic load (GL)

4.12.2 Effect of supplementation of minor millet bran incorporated designer muffins and buns in adolescents

4.12.2.1 Characteristics of the subjects

4.12.2.2 Blood glucose levels

4.12.2.3 Blood lipid profile

4.12.2.4 Blood protein and albumin content

4.12.2.5 Stress profile

4.12.2.5.1 Lipid peroxidation

4.12.2.5.2 Glutathione reductase

4.12.2.5.3 RBC catalase

4.13 Feedback about the products by subjects during 45 days of consumption

4.1 Bran extraction

Numerous grain processing techniques results in removal of bran, seed coat, husk such as milling, dehulling, debranning, polishing etc. (Sikwese and Duodu, 2007; Lohani *et al.* 2012; Devisetti *et al.* 2014 and Palaniappan *et al.* 2017). Based on final intended usage of grains one of these processes are employed. Majority of millets are dehulled or milled before consumption resulting in bran removal. In the present study, stone abrasive dehulling machine was used to dehull grains and obtain millet brans.

It was observed that (Table 4.1) bran yield, dehulling per cent and degree of dehulling varied in each grain. Kodo had significantly highest ($p<0.05$) per cent bran recovery (17.93 ± 1.68), but dehulling per cent (72.21 ± 3.63) and degree of dehulling

(62.95 ± 6.89) were significantly lowest (p>0.05) compared to other grains. After kodo, foxtail millet exhibited higher per cent bran recovery (14.37 ± 1.10) followed by barnyard (12.47 ± 1.36) and proso (12.35 ± 0.69). These differences in bran yield, per cent dehulling and degree of dehulling are associated with grain size, type, moisture levels, dehulling time and equipment used (Mohapatra and Bal, 2010).

Kodo millet being a caryopsis-type, resulted in lower degree of dehulling and dehulling per cent, showing an incomplete dehulling. The pericarp of kodo millet is firmly attached to the seed, making its removal difficult. Other millets such as proso, foxtail and barnyard are utricle type, where the seed coat is loosely attached only at one point facilitating easy removal (Serna-Saldivar and Espinosa-Ramırez, 2019). Despite the lowest per cent dehulling and degree of dehulling, bran yield was highest in kodo than other grains, due to its grain structure (p<0.05). Kodo is reported to have almost 37 per cent bran and husk contributing its total grain weight, while foxtail, proso and barnyard contributes only 13.5 – 23 per cent of total grain weight (Malleshi and Hadimani, 1994, Pawar and Machewad, 2006, Taylor and Emmambux, 2008, Dharmaraj *et al.*, 2016, Sharma and Niranjan, 2018 and Serna-Saldivar and Espinosa-Ramırez, 2019). The yield of dehulled millets and bran recovery mainly depends on the type of dehuller used. It was noted in a previous study that dehulling with centrifugal dehuller resulted in more kernel recovery (up to 10 %) and less broken grains as compared to dehulling in abrasive dehuller (Durairaj *et al.* 2019). Thus, it can be stated that use of abrasive dehuller in present study yielded higher amount of bran from all millets, including husk fractions and ground broken grains.

Table 4.1. Dehulling percentage of millet grains and bran

Millet grains	PBR (%)	Per cent Dehulling	DD (%)
Kodo *(Paspalum scrobiculatum)*	17.93 ± 1.68^{c}	72.21 ± 3.63^{a}	62.95 ± 6.89^{a}
Proso *(Panicum miliaceum)*	12.35 ± 0.69^{a}	83.89 ± 1.27^{c}	80.23 ± 1.80^{c}
Foxtail (*Setaria italica*)	14.37 ± 1.10^{b}	74.68 ± 3.93^{a}	64.42± 7.98^{ab}
Barnyard (*Echinochloa esculenta*)	12.47 ± 1.36^{a}	77.69 ± 2.42^{b}	68.34 ± 4.47^{b}
SEm ±	0.40	0.95	1.83

Note: All values are expressed as Mean ± SD; Per cent bran recovery (PBR), Degree of dehulling (DD). Mean represented within same column having different alphabet show statistically significant difference at 5% (p<0.05)

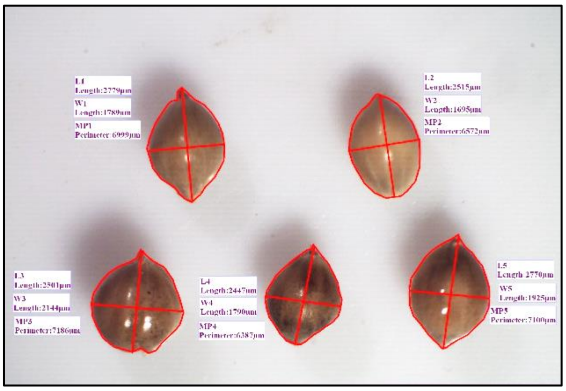
4.2 Grain dimensions and digital image capture

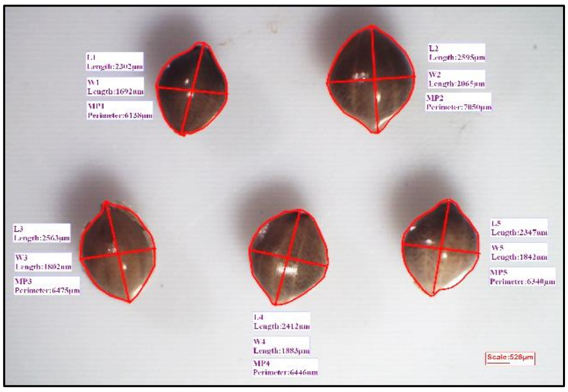
The grain images were captured and dimensions (whole and dehulled grains) were recorded using a stereozoom microscope and reported as average of 10 grains (Table 4.2, Fig. 4.1 – 4.4). It is a known fact that dehulling considerably reduces the grain size as it results in removal of husk, bran and bran rich fractions. Due to minor millets' tiny grain size, a more significant portion is lost during processing (Gulia *et al.* 2007 and Suma and Urooj, 2012). Paired t-test indicated that in all grain dehulling significantly ($p<0.05$) reduced grain size (length, width and perimeter). Only in proso grain width did not reduce significantly ($p>0.05$) after dehulling. The per cent reduction (%) was highest in kodo millet for length (36.35 %), width (19.80 %) and perimeter (25.26 %) this is attributed to its grain structure having more husk/bran, followed by barnyard millet (length: 35.10 %, width: 13.67 % and perimeter: 20.72 %) which is reported to have 23 per cent of husk / bran / glume (Malleshi and Hadimani, 1994; Pawar and Machewad, 2006; Taylor and Emmambux, 2008, Dharmaraj *et al.*, 2016; Sharma and Niranjan, 2018 and Serna-Saldivar and Espinosa-Ramırez, 2019). Foxtail millet showed 32.34 %, 9.74 % and 18.53 % reduction in length, width and perimeter respectively, whereas lowest size reduction viz., 28.51 %, 4.68 % and 14.50 % respectively was observed in proso. Results indicate the degree of dehulling and efficiency of dehuller affects size reduction in grain.

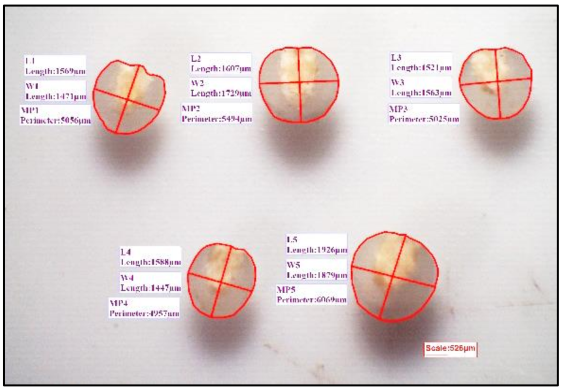
Table 4.2. Grain dimension before and after dehulling

Grains	Kodo		Proso		Foxtail		Barnyard	
Parameters	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**
Length (mm)	2.99 ± 0.267	1.9 ± 0.167	3.13 ± 0.122	2.23 ± 0.119	2.51 ± 0.097	1.70 ± 0.070	2.52 ± 0.160	1.64 ± 0.132
R (%)	36.35		28.51		32.34		35.10	
t-stat	14.781*		14.013*		21.605*		15.084*	
Width (mm)	2.45 ± 0.126	1.97 ± 0.201	2.10 ± 0.155	2.00 ± 0.120	1.59 ± 0.063	1.44 ± 0.082	1.86 ± 0.148	1.61 ± 0.134
R (%)	19.80		4.68		9.74		13.67	
t-stat	7.046*		1.579		4.731*		4.341*	
Perimeter (mm)	8.53 ± 0.551	6.37 ± 0.480	8.18 ± 0.266	7.00 ± 0.202	6.33 ± 0.186	5.16 ± 0.178	6.67 ± 0.376	5.29 ± 0.359
R (%)	25.26		14.50		18.53		20.72	
t-stat	11.498*		9.966*		16.599*		9.792*	

Note: All values are expressed as Mean ± SD; whole (W) and dehulled (D) grains; Reduction (R). (t-critical: 2.262), * indicates significant difference at 5%


a1


a2


b1

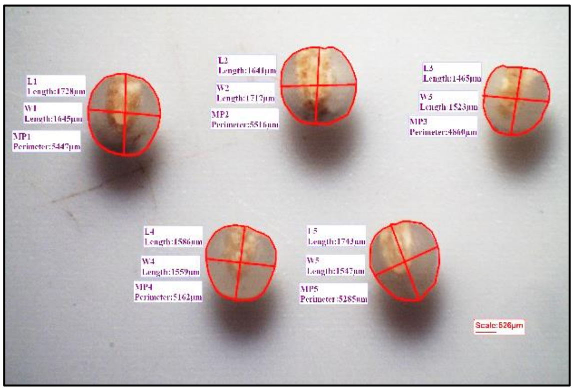
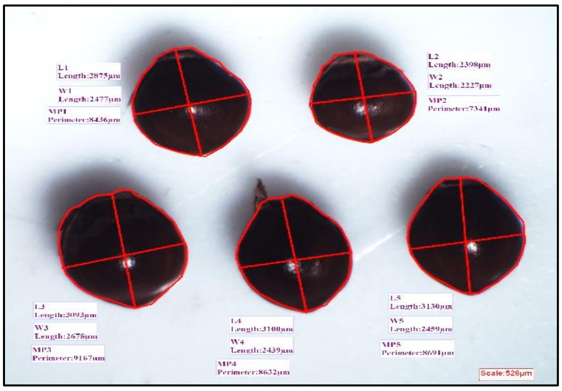
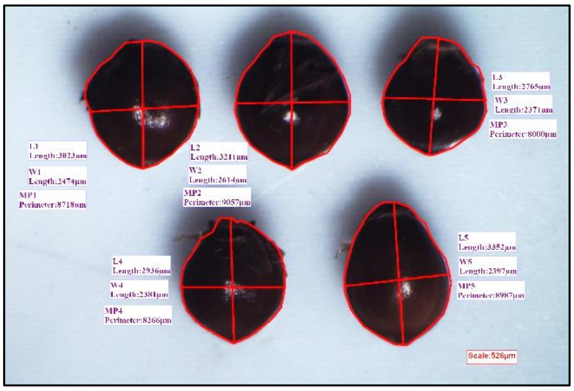
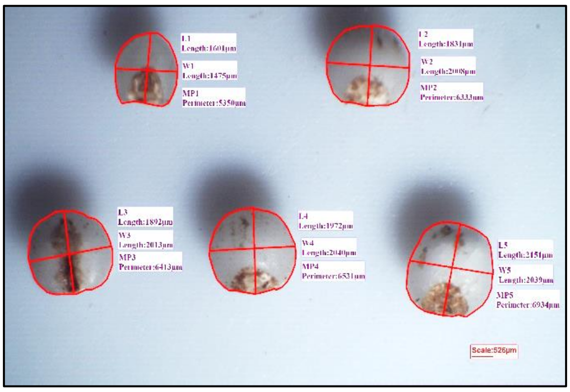

b2

Fig 4.1. Grain dimension of whole (a1, a2) and dehulled (b1, b2) barnyard grain


a1


a2


b1

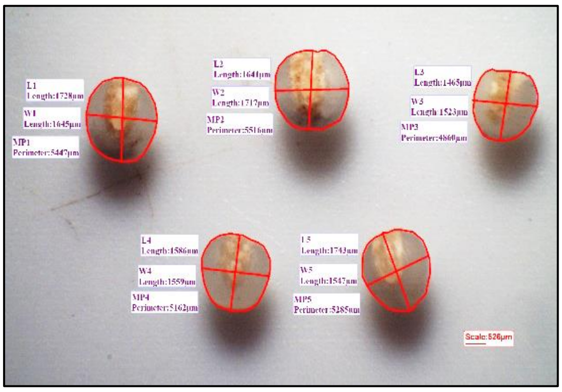
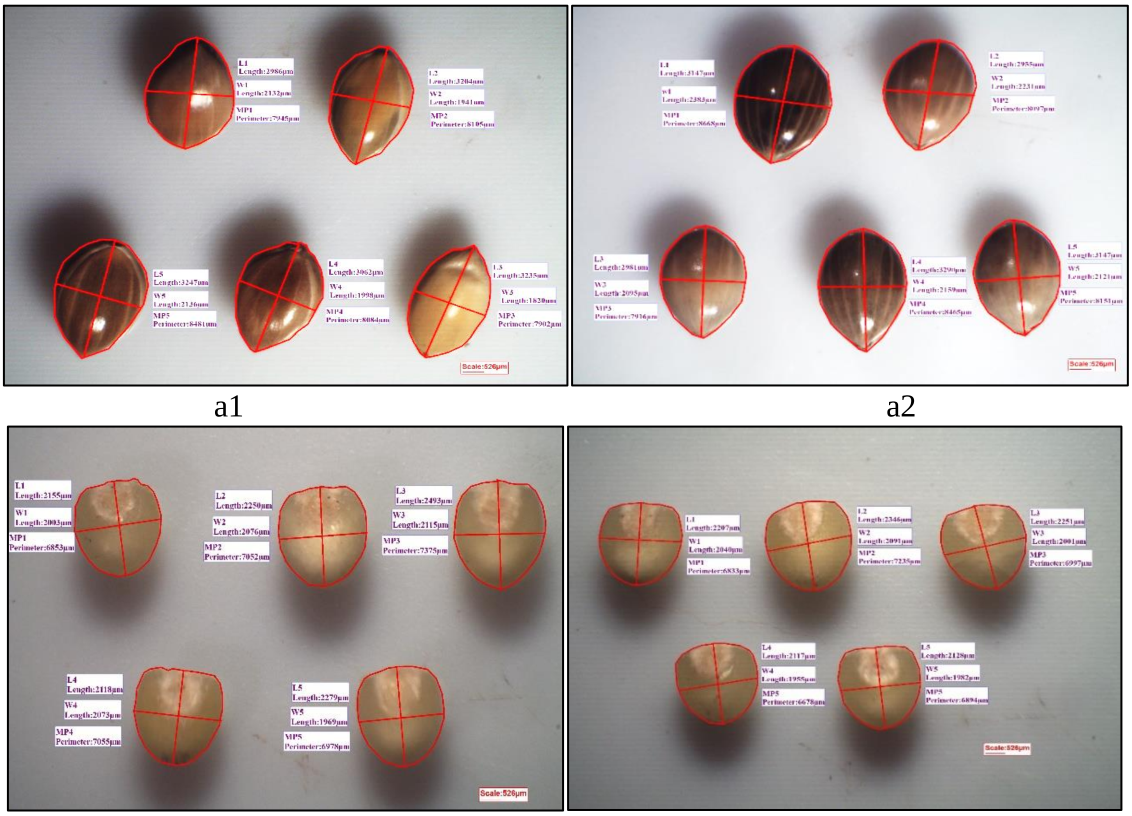

b2

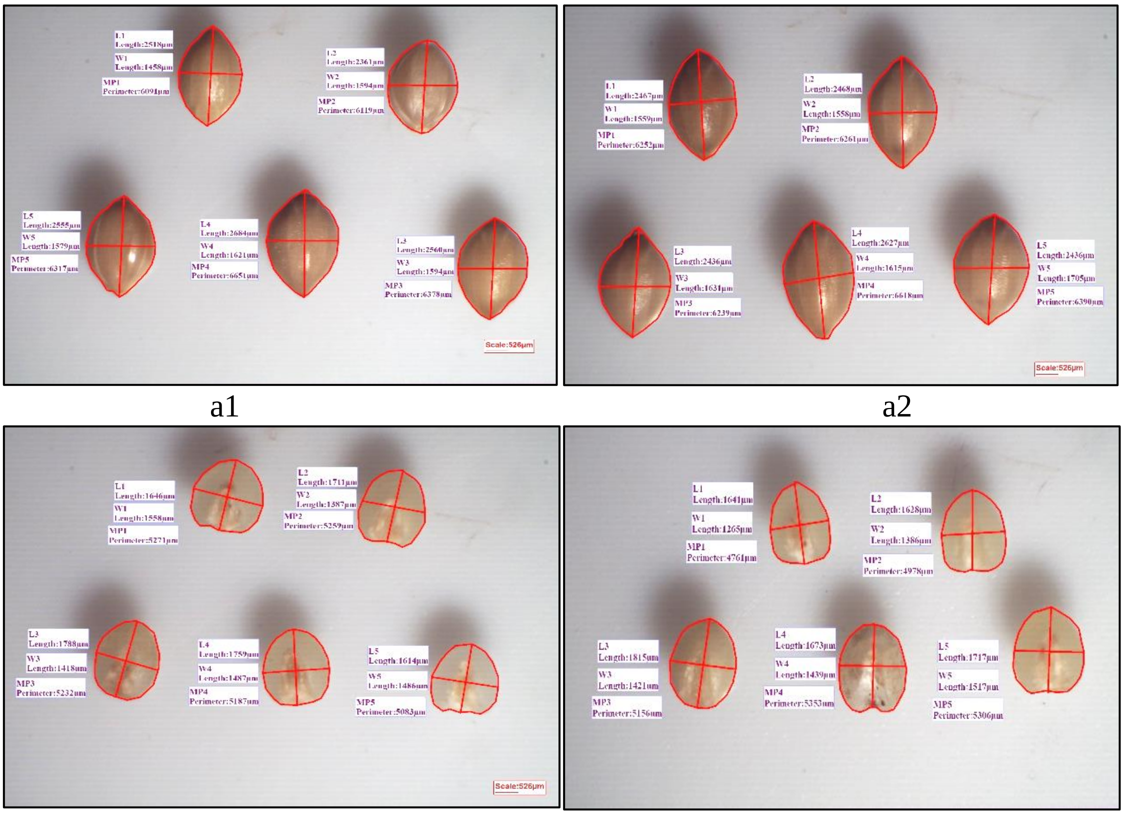
Fig 4.2. Grain dimension of whole (a1, a2) and dehulled (b1, b2) kodo grain



a1 a2

b1 b2

Fig 4.3. Grain dimension of whole (a1, a2) and dehulled (b1, b2) proso grain



a1 a2

b1 b2

Fig 4.4. Grain dimension of whole (a1, a2) and dehulled (b1, b2) foxtail grain

4.3 Stabilization of minor millet brans

Stabilization is a critical step in improving the shelf life of bran. In this study hot air and microwave heating were used to stabilize bran. To assess the effect of stabilization treatments across 15 days of storage (0, 7, 15 days) samples were further analyzed for its moisture and FFA (% oleic acid) content and expressed as mean ± SD in Table 4.3.

Table 4.3. Mean FFA and moisture scores of minor millet brans during storage

	Free Fatty Acid (FFA)			Moisture		
	0 day	7 day	15 day	0 day	7 day	15 day
Foxtail Millet Bran						
CT	5.21±0.57	9.19±0.00	10.33±0.92	6.40 ±0.23	7.12 ±0.31	7.51 ±0.26
HT1	3.41±0.33	3.79 ± 0.37	3.86 ± 0.16	1.69 ±0.09	1.84 ±0.11	1.98 ±0.01
HT2	3.08±0.57	3.27±0.14	3.80 ± 0.42	1.81 ±0.06	1.93 ± 0.06	2.03 ±0.02
MW1	3.11±0.12	3.81±0.10	3.90 ± 0.26	3.54 ±0.25	3.75 ± 0.20	3.81 ±0.08
MW2	2.99±0.34	3.74±0.60	3.89 ± 0.33	2.38 ±0.07	2.39 ±0.07	2.42 ±0.04
Barnyard Millet Bran						
CT	4.42 ±0.10	8.24 ±0.11	10.47±1.08	5.67 ±0.13	5.83 ±0.14	6.13 ±0.04
HT1	3.36 ±0.20	3.64 ±0.19	3.80 ±0.42	1.17 ±0.14	1.19± 0.14	1.20 ±0.13
HT2	3.40 ±0.26	3.92 ±0.11	3.97 ±0.47	1.20 ±0.13	1.22± 0.14	1.24 ±0.13
MW1	3.89 ±0.27	3.93 ±0.05	4.02 ±0.06	2.95 ±0.18	3.04± 0.14	3.07 ±0.13
MW2	3.86 ±0.22	3.90 ±0.06	3.97 ±0.17	2.40 ±0.11	2.41± 0.11	2.44 ±0.10
Proso Millet Bran						
CT	1.11 ±0.04	20.21±0.46	26.88±0.15	6.83 ±0.16	7.01 ±0.12	7.84 ±0.50
HT1	3.28 ±0.06	4.68 ±0.48	4.95 ±0.78	1.44 ±0.12	1.50 ± 0.13	1.53 ±0.12
HT2	3.09 ±0.38	5.84 ±0.80	6.71 ±1.02	2.76 ±0.01	2.80 ± 0.03	2.83 ±0.02
MW1	3.01 ±0.32	4.51 ±0.74	7.76 ±0.82	3.47 ±0.02	3.51 ± 0.04	3.58 ±0.01
MW2	3.06 ±0.31	3.00 ±0.10	4.34 ±0.54	1.87 ±0.04	1.89 ± 0.08	1.91 ±0.08
Kodo Millet Bran						
CT	2.65 ± 0.13	12.20±0.16	21.39±5.24	6.19 ±0.12	6.72 ± 0.09	7.00 ±0.19
HT1	2.14 ± 0.22	3.71±0.54	5.13 ±0.06	1.23 ±0.10	1.26 ± 0.10	1.29 ±0.12
HT2	3.18 ± 0.22	3.67 ±0.31	4.33 ±0.28	2.00 ±0.15	2.05 ± 0.12	2.11 ±0.08
MW1	1.56 ± 0.28	4.07±0.13	6.97 ±0.94	3.46 ±0.02	3.53 ± 0.03	3.57 ±0.03
MW2	2.62 ± 0.59	3.14 ± 0.11	4.78 ±0.37	1.75 ±0.18	1.80 ± 0.18	2.00 ±0.09

Note: Values expressed as mean±SD; CT- Control; Hot air oven treatment: HT1- 100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave heating: MW1-1.5 minutes at 900 W, MW2: for 2.5 minutes at 900 W; Storage days are 0, 7 and 15.

4.3.1 Effect of treatment, storage and grain on bran moisture

Moisture content increased during storage for all the treated as well as control brans due to their hygroscopic nature. Multifactor ANOVA revealed that there was a significant effect of ($p<0.01$) treatment, storage and grain on moisture (Table 4.4 – 4.5; Fig 4.5 and 4.6). Irrespective of brans, the interaction between treatment and storage revealed that CT had highest percentage increase of moisture (13.55 %), followed by HT1 (8.69 %), HT2 (5.67 %), MW1 (4.46 %) and MW2 (4.28 %) (Fig 4.6). On the 0 day, hot air oven treatments (HT1, HT2) had less moisture content compared to microwave treatments (MW1, MW2), the lower moisture for HT1 and HT2 could be due to more exposure time while heating in hot air oven. But during the storage period per cent increase in moisture content was higher in hot air oven treatments than in microwave treatments, suggesting that microwave treatments were more suitable for stabilization. Further the least increase was observed in MW2 indicating that it was best suited in controlling moisture increase during storage periods. Several authors have also reported microwave treatment is best for stabilization of bran (Bagchi *et al.* 2014 and Patil *et al.* 2016).

4.3.2 Effect of treatment, storage and grain on bran FFA

Increase in FFA during storage period was noted for all brans (treated and control) and was highest, rapid in control than treated samples. There was sharp increase of 10.33 per cent, 10.47 per cent, 21.39 per cent and 26.88 per cent after 15th day of storage in control samples of foxtail, barnyard, kodo and proso millet brans respectively making it unfit for consumption (Table 4.3). FFA during storage below 10 per cent in millet flour, rice bran oil and below 5 per cent for rice bran are acceptable for human consumption (Tao *et al.* 1993 and Rani *et al.* 2018).

In HT1 mean increase during 0 to 15 days was 3.41 to 3.86 per cent (Foxtail bran), 3.28 to 4.95 per cent (Proso bran), 2.14 to 5.13 per cent (Kodo bran) and 3.36 to 3.80 per cent (Barnyard bran). Similarly, in HT2 FFA raised from 3.08 to 3.80 per cent, 3.09 to 6.71 per cent, 3.18 to 4.33 per cent and 3.40 to 3.97 per cent for foxtail, proso, kodo, barnyard bran respectively (Table 4.3). In microwave stabilized bran FFA levels increased from 3.11 to 3.90 per cent (MW1) and 2.99 to 3.83 per cent (MW2) for foxtail bran, whereas in barnyard bran it was from 3.89, 3.86 per cent (MW1, MW2) at 0 day to 4.02, 3.97 per cent respectively at 15th day (Table 4.3). Higher increase of FFA was observed in proso and kodo compared to foxtail and barnyard bran at 15th day of storage.

This increase in FFA could be attributed to higher lipase activity, hygroscopic nature of bran leading to absorption of moisture and increase oxidation of millet bran oil (Daud *et al.* 2017).

Among the treatments there was no significant difference ($p<0.01$) between both the hot air oven treatments, HT1× MW2 and HT2 × MW1 (Table 4.4 – 4.5; Fig 4.7 and 4.8). It was observed that MW2 had the least increase in FFA, followed by HT1, HT2 and MW1. Interaction plot for all grains and treatments suggested that MW2 was most effective stabilization treatment for all the grains maintaining FFA below 5 per cent followed by HT1, HT2 and MW1. This could be due to the high-power exposure, uniform internal heating of bran in microwave compared to the external heating in hot air oven (Tao, 1989). These results agree with Bagchi *et al.* (2014) where microwave stabilization (900 W for 2.5 min) was found to be best for rice bran. Stabilization of rice and other cereal brans, using microwave heating was found useful (Ramezanzadeh *et al.* 2000, Lakkakula *et al.* 2004, Sharma *et al.* 2004, Sairam *et al.* 2011, Bhosale and Vijayalakshmi, 2015, Patil *et al.* 2016 and Daud *et al.* 2017) as it was less cumbersome, retains nutrients and controls rapid FFA formation thus, increasing the storage stability.

Both hot air oven treatments (HT1, HT2) were also suitable for stabilization followed by microwave treatments. At industrial levels where stabilization with microwave oven is not possible considering its huge initial capital investment, hot air oven treatment can be utilised. Suitability of hot air oven for stabilisation are presented in several studies (Sudha *et al.* 2011 and Lv *et al.* 2018).

Results in the present study indicate that both microwave and hot air oven stabilized minor millet brans could be stored safely for 15 days without the signs of spoilage like rancidity or off flavor. This was possible as stabilization treatments were able to evaporate the moisture molecules resulting in lesser production of FFA and hydrolysis of oil during storage.

Table 4.4. ANOVA table for Free Fatty Acids (FFA) (% oleic acid) content and moisture as affected by different factors

Source	Degree of Freedom	Sum of Squares		Mean Square		F-Ratio		P-Value	
		FFA	Moisture	FFA	Moisture	FFA	Moisture	FFA	Moisture
Main Effects									
A: Treatment	4	1464.27	642.83	366.06	160.70	564.96	7686.53	0.0000***	0.0000***
B: Grain	3	158.07	12.45	52.69	4.15	81.32	198.65	0.0000***	0.0000***
C: Storage (Days)	2	521.89	2.06	260.94	1.03	402.73	49.38	0.0000***	0.0000***
Interactions									
AB	12	282.10	15.53	23.50	1.29	36.28	61.91	0.0000***	0.0000***
AC	8	758.17	2.58	94.77	0.32	146.26	15.47	0.0000***	0.0000***
BC	6	221.48	0.30	36.91	0.05	56.97	2.43	0.0000***	0.0285*
ABC	24	335.57	3.01	13.98	0.02	21.58	7686.53	0.0000***	0.0000***
Residual	120	77.75	678.80	0.64	160.70				
Total (Corrected)	179	3819.32	15.53						

Note: All F-ratios are based on the residual mean square error. Significant difference is denoted by * (p<0.05), ** (p<0.01), ***(p<0.001). In the tables grain indicate respective grains from which bran was obtained

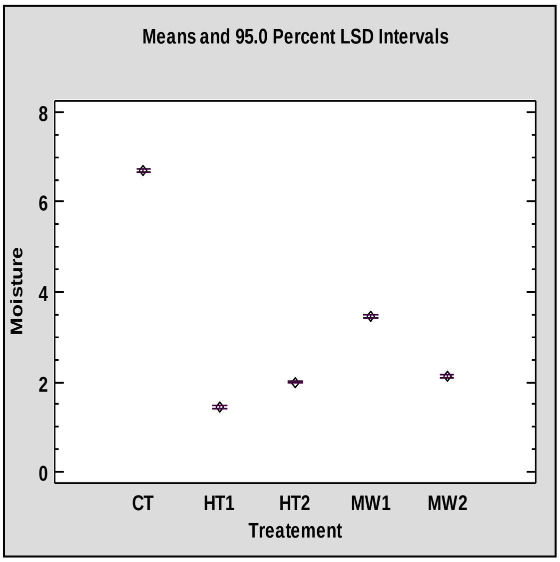
Table 4.5. Main effects of treatment, grain and storage on FFA (% oleic acid) and moisture content of minor millet brans

Treatment			Grain			Storage (days)		
Grand Mean			Grand Mean			Grand Mean		
	FFA	Moisture		FFA	Moisture		FFA	Moisture
CT	11.03^{d}	6.69^{e}	BM	4.59^{a}	2.74^{a}	0	3.12^{a}	3.01^{a}
HT1	3.81^{ab}	1.44^{a}	FM	4.49^{a}	3.37^{c}	7	5.62^{b}	3.14^{b}
HT2	4.02^{bc}	2.00^{b}	KM	5.44^{b}	3.06^{b}	15	7.26^{c}	3.27^{c}
MW1	4.21^{c}	3.44^{d}	PM	6.83^{c}	3.39^{c}	SEm ±	0.10	0.01
MW2	3.61^{a}	2.14^{c}	SEm ±	0.11	0.02			
SEm ±	0.13	0.02						

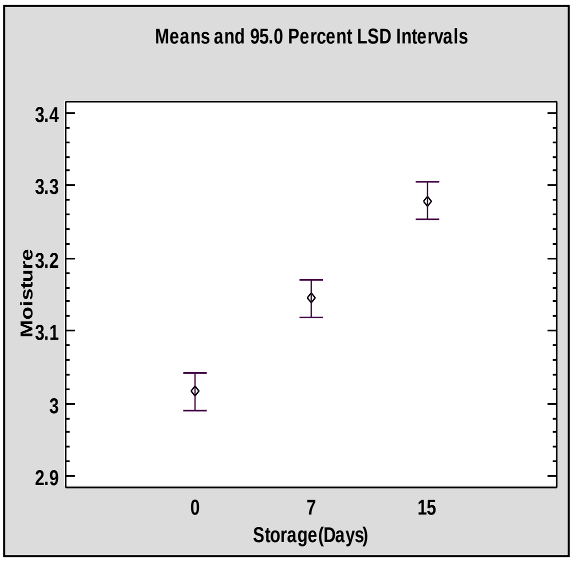
Note: Mean ± SEm represented within same column having different alphabet show statistically significant difference at 1% (p<0.01). CT- Control, Hot air oven heating: HT1-100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave oven heating: MW1-1.5 minutes at 900 W, MW2- 2.5 minutes at 900 W. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Storage days are 0, 7 and 15. In the table grain indicates respective grain from which bran was obtained.

4.4 Functional properties of minor millet brans

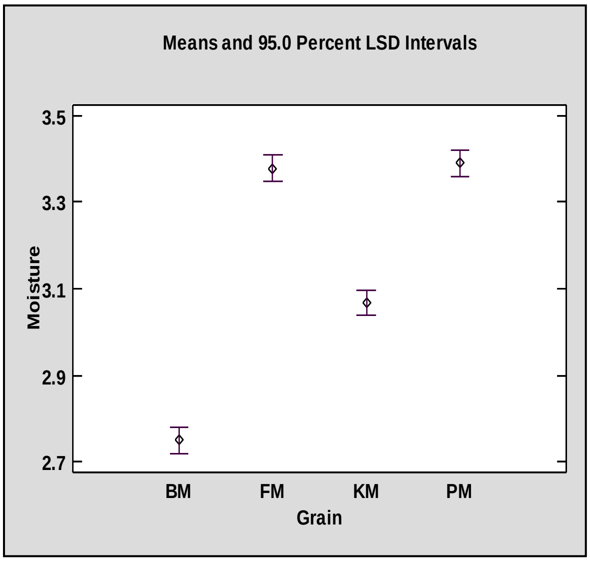
Water absorption, oil absorption and foaming capacity were estimated for all stabilized brans (MW2). The results are presented in Table 4.6. It was observed that bran had good water absorption capacity due to its hygroscopic nature. A significant difference ($p<0.05$) was noted in water absorption capacity among all brans. Amongst all the brans highest water absorption capacity was found in barnyard and foxtail millet bran with no significant difference. Least water absorption was observed in kodo bran (246.47 ml/100 g). This variation in water absorption capacity amongst all bran could be associated with the variation in pH, starch, protein, lipids and dietary fiber content. It was noted through various studies that high proteins, soluble fibers, presence of polysaccharides and low lipid content might enhance the water absorption (Chaplin, 2003; Sairam *et al.* 2011 and Dhankhar *et al.* 2019). Water absorption in all brans ranged from 246.47 to 298.42 ml/100 g. These results agree with those of stabilized rice brans (Sairam *et al.* 2011). Similarly, oil absorption ranged from 162.62 – 258.80 g/100 g and differed significantly amongst the brans ($p<0.05$). Barnyard millet bran had the highest OAC value, followed by kodo, foxtail and proso millet. Present results indicated that OAC of minor millet brans are comparable to OAC of other cereal brans as reported by Bhosale and Vijayalakshmi (2015). These functional properties are essential in food industry during food formulation. A good oil absorption helps to retain the product's flavour and contributes to the mouthfeel (Ahmad *et al.* 2015). Good water retention is desired where moisture retention is essential in final product. Brans exhibited no foaming and this result correlated with a study where extruded full fat rice bran showed no or little foaming capacity (James and Sloan 1984 and Luh *et al*. 1991).



a) Effect of treatment on moisture



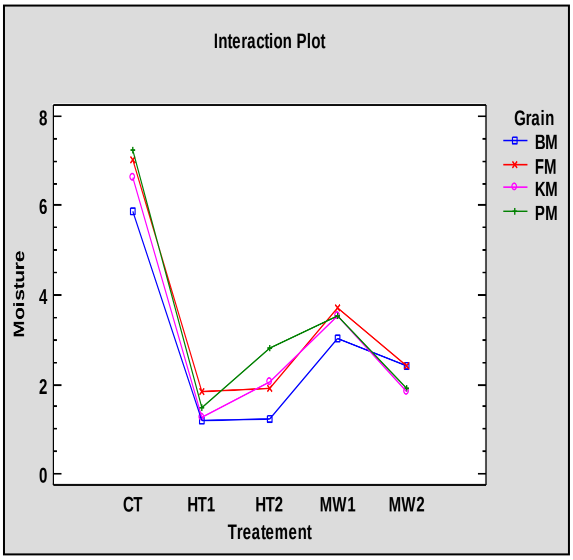
b) Effect of storage on moisture



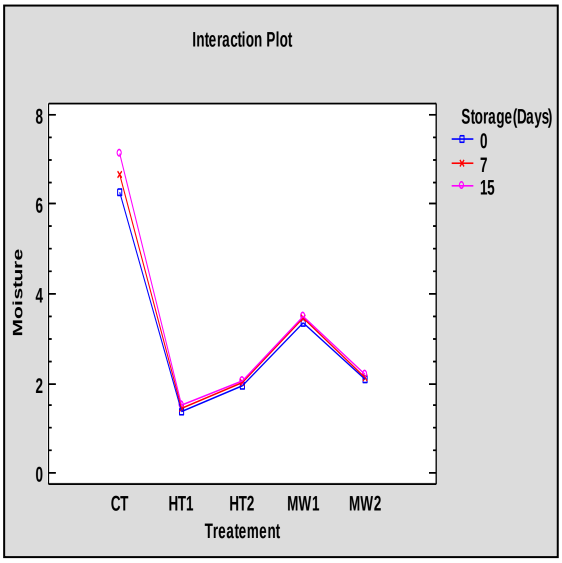
c) Effect of grain on moisture

Fig 4.5 Effect of treatment, storage and grain on moisture

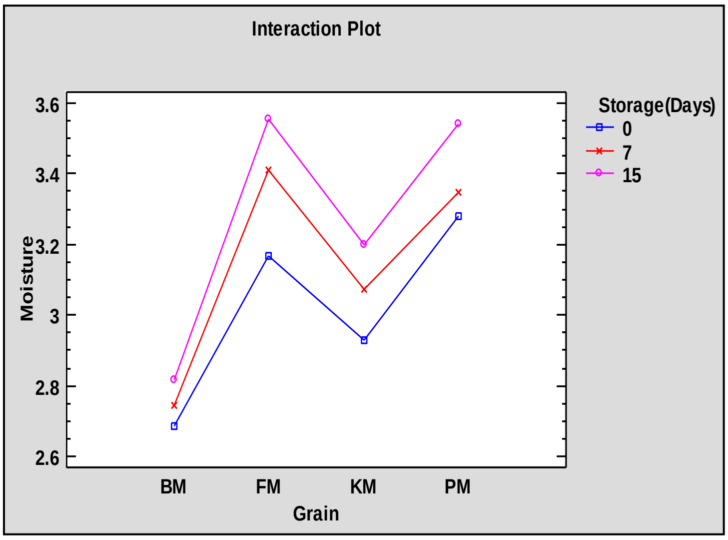
Note: CT- Control, Hot air oven heating: HT1-100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave oven heating: MW1-1.5 minutes at 900 W, MW2- 2.5 minutes at 900 W. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Storage days are 0, 7 and 15 ($p<0.01$). In the figure grain indicates respective grain from which bran was obtained



a) Treatment by grain



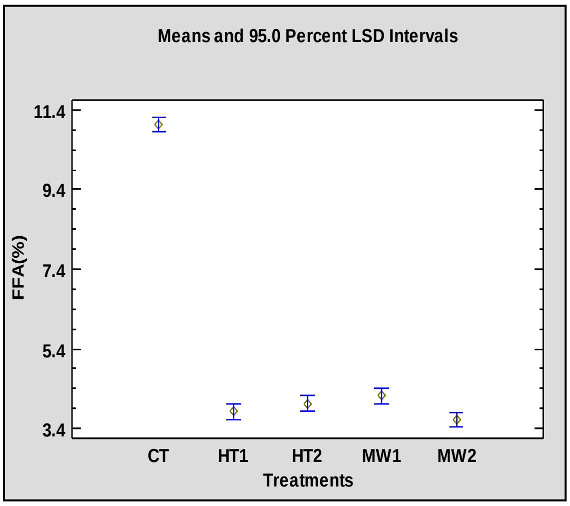
b) Treatment by storage



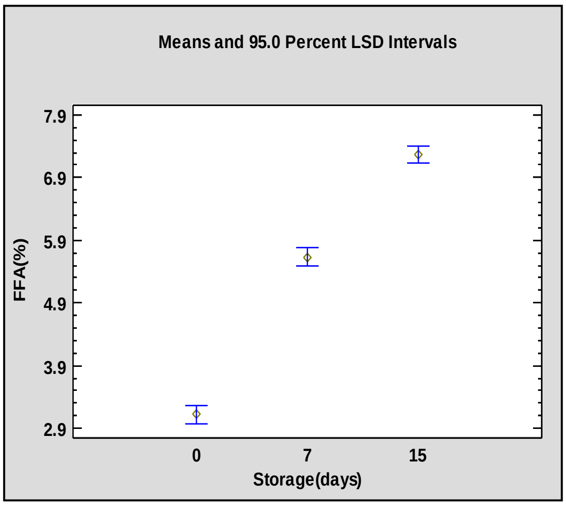
c) Grain by storage

Fig 4.6 Interaction effect of treatment, grain and storage (days) on moisture

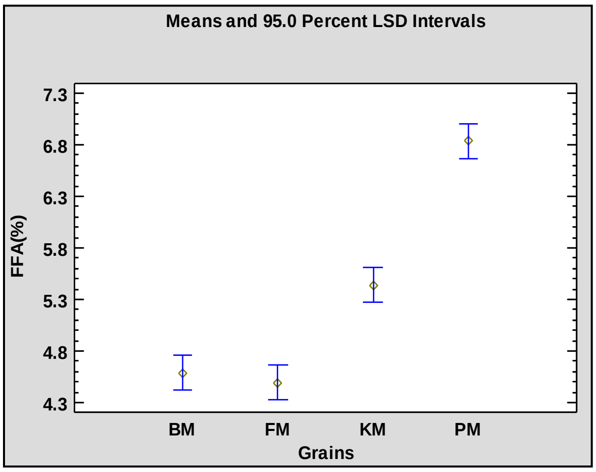
Note: CT- Control, Hot air oven heating: HT1-100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave oven heating: MW1-1.5 minutes at 900 W, MW2- 2.5 minutes at 900 W. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Storage days are 0, 7 and 15 ($p<0.01$). In the figure grain indicates respective grain from which bran was obtained



a) Effect of treatment on FFA



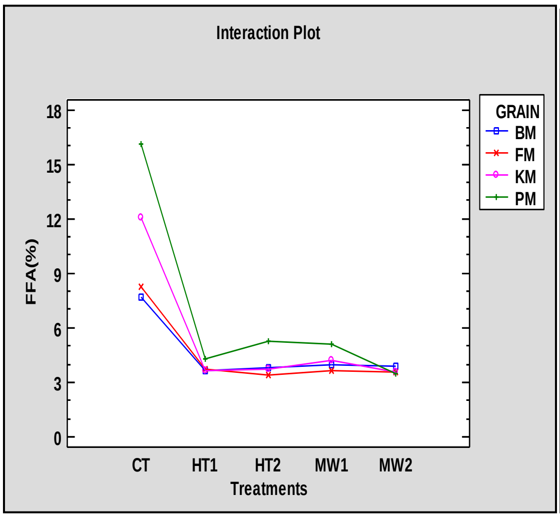
b) Effect of storage on FFA



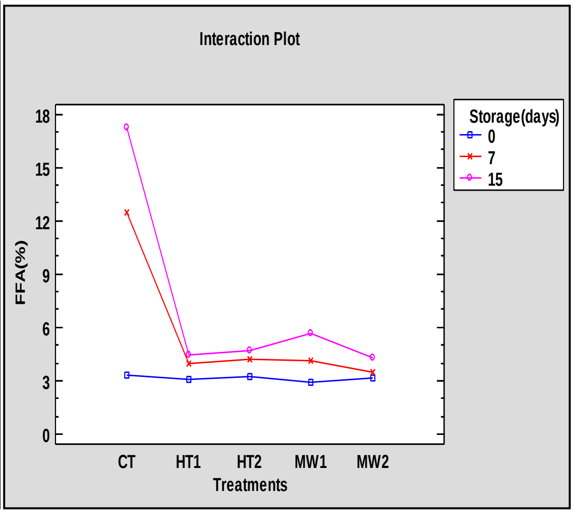
c) Effect of Grain/Bran on FFA

Fig 4.7 Effect of treatment, storage and grain on FFA

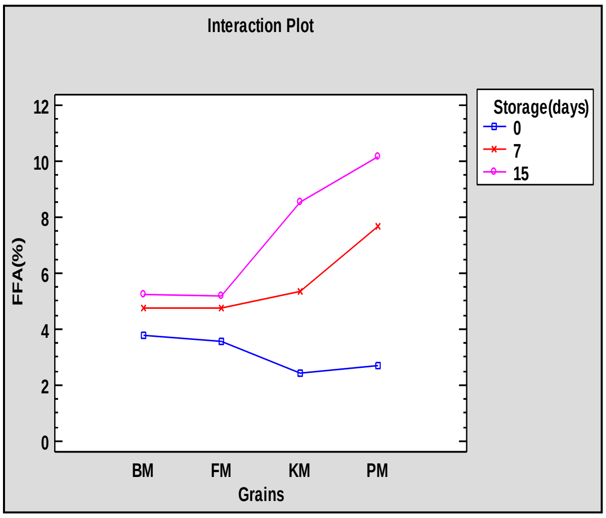
Note: CT- Control, Hot air oven heating: HT1-100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave oven heating: MW1-1.5 minutes at 900 W, MW2- 2.5 minutes at 900 W. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Storage days are 0, 7 and 15 ($p<0.01$). In the figure grain indicates respective grain from which bran was obtained



a) Treatment by grain



b) Treatment by storage



c) Grain by storage

Fig 4.8 Interaction effect of treatment, grain and storage (days) on FFA

Note: CT- Control, Hot air oven heating: HT1-100ºC for 3 hours, HT2- 130ºC for 20 minutes; Microwave oven heating: MW1-1.5 minutes at 900 W, MW2- 2.5 minutes at 900 W. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Storage days are 0, 7 and 15 ($p<0.01$). In the figure grain indicates respective grain from which bran was obtained

Table 4.6. Functional properties of stabilized minor millet brans

Millet brans	WAC (g/100 g)	OAC (g/100 g)	FC (%)
FSM	297.52^{b}	181.37^{b}	0.26^{NS}
BSM	298.42^{b}	258.80^{d}	0.13^{NS}
PSM	248.51^{a}	162.62^{a}	0.16^{NS}
KSM	246.47^{a}	215.65^{c}	0.20^{NS}
SEm±	0.71	2.35	0.12

Note: Means ± SEm within same column having different alphabet show statistically significant difference at 5 %. NS indicates no significant difference. FSM: Foxtail millet bran, KSM: Kodo millet bran, PSM: Proso millet bran, BSM: Barnyard millet bran. Water absorption capacity (WAC), Oil absorption capacity (OAC) and Foaming capacity (FC).

4.5 Nutrient profile of minor millet brans

Nutritional parameters were assessed for all the stabilised minor millet brans (MW2) and results were tabulated in Table 4.7 – 4.8 for proximate and mineral content respectively.

4.5.1 Proximate content

It was observed that there was no significant difference between proso and kodo concerning moisture content. Similarly, foxtail and barnyard did not differ significantly ($p>0.05$). The moisture content of brans ranged from 1.76 – 2.41 g/100 g, that was low when compared to previous reports by Liu *et al.* (2012) and Bisoi *et al.* (2012) for unstabilised proso (7.32 g/100 g), kodo (5.53 g/100 g), foxtail (9.07 g/100 g) and barnyard (6.79 g/100 g) brans. The lower values in present study might be due to evaporation of moisture during microwave stabilisation. The results agree with previous reports where, microwave stabilisation reduced moisture content (Bagchi *et al.* 2014 and Patil *et al.* 2016).

Amongst all the brans, protein content was highest in PSM (13.04 g/100 g) followed by FSM (10.49 g/100 g), BSM (7.70 g/100 g) and least were reported in KSM (5.68 g/100 g). These values agree with the range of values reported in previous studies (Liang *et al.* 2010; Bisoi *et al.* 2012; Sarma *et al.* 2017 and Mustač *et al.* 2020).

Fat content in brans ranged from 5.28 to 9.87 g/100 g, with FSM having the highest and KSM lowest values (Table 4.7). Amongst all the brans BSM and PSM exhibited no significant variation and values were 7.85 and 7.13 g/100 g respectively. The present fat values for foxtail bran are within the range of previously reported values *i.e.*, 5.65 – 9.63 %. But the results for other brans viz., barnyard, proso and kodo are slightly higher than the previously reported fat values (Liang *et al.* 2010; Amadou *et al.* 2011; Liu *et al.* 2012; Bisoi *et al.* 2012; Nazni and Karuna, 2016 and Sarma *et al.* 2017).

The ash content of brans also differed significantly ($p<0.05$) with highest content in FSM, followed by BSM, PSM and KSM. All these differences in the composition of bran in the present and previous research can be attributed to genetic variation, environmental and storage conditions, processing of grain *i.e.*, degree of dehulling, milling, polishing etc. (Abdul-Hamid *et al.* 2007).

The crude fiber (CF) content was highest in KSM (32.52 g/100 g) followed by PSM (31.94 g/100 g), FSM (29.51 g/100 g) and BSM (29.34 g/100 g). A significant difference ($p<0.05$) existed among KSM, FSM and BSM, but no difference was observed between BSM and FSM. Slightly higher CF values were reported for foxtail (42.56 – 51.69 %), kodo (34.24 %), proso (31.63 %) and barnyard (38.4 – 45.10 %) brans (Liang *et al.* 2010; Amadou *et al.* 2011; Bisoi *et al.* 2012; Nazni and Karuna, 2016 and Sarma *et al.* 2017). KSM had significantly highest total dietary fiber (TDF) content (61.52 %) compared to other brans, followed by BSM (37.26 %), PSM (34.74 %) and FSM (34.39 %). On the contrary, a previous study reported higher dietary fiber for foxtail (60.69 %) bran (Zhu *et al.* 2018). This difference in the values could be attributed to varietal difference in grains used in both study and also sample preparation. In the present study full fat bran samples were used while defatted samples in previous study (Zhu *et al.* 2018). In the present study kodo bran had higher TDF value (Sarma *et al.* 2017) and proso had similar value (Mustač *et al.* 2020) compared to previous studies. These findings suggest that variations in dietary fiber composition can be mainly due to use of bran obtained from different varieties and sample processing like defatting, reducing the particle size or micronisation.

Higher TDF content in KSM also resulted in reduction in available CHO and energy (Table 4.7). Total carbohydrate content in all brans ranged from 65.10 – 80.39 g/100 g with FSM and KSM having lowest and highest values respectively. The available CHO ranged from 18.86 – 34.90 g/100 g with KSM having lowest and PSM having highest values (Table 4.7). CF and TDF content depict that the brans are good source of fiber. According to Indian recommended dietary allowances (RDA) an adult should consume 40 g of dietary fibre (based on 2000 Kcal diet) per day (ICMR, 2020), consumption of whole grains, fruits, leafy vegetables etc. are promoted to meet these requirements. These dietary fibers contribute to various health benefits such as reducing glucose, cholesterol levels and they also provide laxative effect (Patel, 2015). Along with all these benefits, dietary fiber's water holding and bulking properties can be exploited in food formulations. Thus, bran can be used in formulating various new products with nutraceutical properties as they are rich in fiber content (Onipe *et al.* 2015).

4.5.2 Mineral content

Calcium, sodium and potassium are some of the important macro minerals whereas iron and zinc are micro minerals that are necessary for body's normal functioning. It was observed that iron, zinc, calcium, sodium and potassium content ranged from 8.87 – 65.58 mg/100 g, 2.13 – 5.59 mg/100 g, 37.87 – 94.63 mg/100 g, 2.23 – 18.08 mg/100 g and 343.85 – 630.83 mg/100 g respectively (Table 4.8). The present study reveals that, mineral content of minor millet brans is comparable to rice and wheat bran (Shenoy and Prakash, 2002; Sairam *et al.* 2011; Brouns *et al.* 2012; El-Sharnouby *et al.* 2012; Curti *et al.* 2013; Bhosale and Vijayalakshmi, 2015; Onipe *et al.* 2015; Zhao *et al.* 2017; Chalamacharla *et al.* 2018 and Dhillon and Tanwar, 2018).

Table 4.7. Proximate content of minor millet brans

Nutrients	FSM	BSM	PSM	KSM	SEm ±
Moisture (g/100g)	2.39^{b}	2.41^{b}	1.88^{a}	1.76^{a}	0.07
Protein (g/100g)	10.49^{c}	7.70^{b}	13.04^{d}	5.68^{a}	0.46
Fat (g/100g)	9.87^{c}	7.85^{b}	7.13^{b}	5.28^{a}	0.43
Ash (g/100g)	12.15^{d}	10.02^{c}	8.32^{b}	6.90^{a}	0.02
CF (g/100g)	29.51^{a}	29.34^{a}	31.94^{ab}	32.52^{b}	0.86
TDF (%)	34.39^{a}	37.26^{b}	34.74^{a}	61.52^{c}	0.16
T. CHO (g/100g)	65.10^{a}	72.03^{c}	69.64^{b}	80.39^{d}	0.70
A. CHO (g/100g)	30.71^{b}	34.78^{c}	34.90^{c}	18.86^{a}	0.77
Energy (Kcal/100g)	391.21^{NS}	389.56^{NS}	394.83^{NS}	391.80^{NS}	2.14

Note: Mean within same row having different alphabet show statistically significant difference at 5%. NS: no significant difference. FSM: Foxtail millet bran, KSM: Kodo millet bran, PSM: Proso millet bran, BSM: Barnyard millet bran.

Iron deficiency anaemia is one of the global problems majorly affecting adolescent girls. Iron is also required for numerous functions in body *i.e.*, mental, physical growth etc. (Kumari *et al.* 2017b). RDA for Indians recommends 19 mg/day and 29 mg/day for adult men and women respectively (ICMR, 2020). The present study revealed that PSM and FSM brans have higher iron content compared to RDA of both adult men and women, whereas and KSM has more than half of RDA; thus, it can be used in food formulations as fortificant. The iron content of brans ranged from 8.87 to 65.58 mg/100 g and was as follows in increasing order BSM (8.87 mg/100 g) < KSM (20.45 mg/100 g) < PSM (60.74 mg/100 g) < FSM (65.58 mg/100 g).

Zinc is another vital micromineral that forms part of more than 300 metalloenzymes and is essential in synthesis, metabolism, degradation of macronutrients, micronutrients and nucleic acids (King and Keen 2003). In the present study it was revealed that PSM (5.59 mg/100 g) had highest content of zinc followed by FSM (4.71 mg/100 g), BSM (3.83 mg/100 g) and KSM (2.13 mg/100 g).

Amongst macro minerals, calcium is abundantly found in human body. It assists in maintaining bones and teeth and is associated with various other vital functions like nerve and muscle impulse, contraction and relaxation of muscles etc. An adequate intake of calcium may reduce risk of obesity, osteoporosis, fractures and diabetes in some populations (Beto 2015). Analysis in the present study revealed that brans are fair source of calcium. The trend was as follows with FSM having highest followed by KSM (76.09 mg/100 g), BSM (62.28 mg/100 g) and PSM (37.87 mg/100 g). Calcium content of minor millet brans in the present study were higher than that of rice bran (Sairam *et al.* 2011; Oluremi *et al.* 2013 and Bhosale and Vijayalakshmi, 2015) and comparable to wheat bran as reported by previous studies (Shenoy and Prakash, 2002; Brouns *et al.* 2012 and El-Sharnouby *et al.* 2012).

Other important macro minerals are sodium and potassium – known as electrolytes of body. Researchers suggest that higher potassium and lower sodium intake can be linked with reduced risk of strokes and hypertension (Green *et al.* 2002; Geleijnse *et al.* 2007; Kong *et al.* 2016 and Xu *et al.* 2017). This study revealed that all brans have higher potassium content and lower sodium content (Table 4.8) thus, it may offer benefit to patients suffering from cardiovascular problems. All these results indicated that brans possess a mineral profile comparable to other cereal brans.

Table 4.8. Mineral content of minor millet brans

Bran	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)
FSM	65.58^{c}	4.71^{c}	94.63^{d}	584.04^{c}	5.12^{b}
BSM	8.87^{a}	3.83^{b}	62.26^{b}	550.21^{b}	18.08^{c}
PSM	60.74^{c}	5.59^{d}	37.87^{a}	630.83^{d}	2.23^{a}
KSM	20.45^{b}	2.13^{a}	76.09^{c}	343.85^{a}	5.20^{b}
SEm ±	2.43	0.06	1.38	2.49	0.21

Note: Means within same column having different alphabet show statistically significant difference at 5%. FSM: Foxtail millet bran, KSM: Kodo millet bran, PSM: Proso millet bran, BSM: Barnyard millet bran

4.6 Antioxidant capacity and phytonutrient content of minor millet brans

It is a well-known fact that millets and cereals have good antioxidant potential and it is mainly attributed to the presence of phenolic compounds concentrated in brans. Thus, contributing antioxidant capacity to brans and bran rich fractions (Suma and Urooj, 2012; Kundgol *et al.* 2013 and Bijalwan *et al.* 2016). Many protocols are employed in estimation of antioxidant, anti-nutritional properties, and many bioactive compounds that contribute to the antioxidant capacity of in brans. In the present study, selected antioxidant and phytonutrient properties were analysed. The results of evaluated bran samples are documented in Table 4.9. Extraction of antioxidants depends on wide range of factors, such as solvents used, polarity of various active components, solubility of the bioactive compounds, stability etc. making it a tedious task. The literature available suggested that methanol is suitable for extraction of antioxidants as it is cheap, easily available and highly efficient (Singh *et al.* 2002 and Suma and Urooj, 2014). Thus, methanol was used as solvent to extract samples for total phenol (TP), flavonoids, DPPH and FRAP analysis.

4.6.1 DPPH radical scavenging activity

DPPH scavenging activity is based on reduction of the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical that is stable at room temperature by the antioxidants present in sample from purple to pale yellow colour. Thus, higher discoloration of DPPH indicates that sample has higher antioxidant capacity. It was observed from the results (Table 4.9) that per cent inhibition was highest in KSM (31.13 %) followed by BSM (15.26 %), FSM (14.06 %) and PSM (12.94 %). FSM, PSM and BSM exhibited similar antioxidant capacity in terms of DPPH ($p<0.05$) while KSM had significantly highest radical scavenging activity compared to other brans ($p<0.05$). The results agree with a study where DPPH assay indicated that hydroxycinnamic acid bound arabinoxylans (HCA-AXs) extracted from kodo bran had lowest EC_{50} value indicating its highest antioxidant potential compared to foxtail, proso, finger and barnyard millet bran HCA-AXs extracts (Bijalwan *et al.* 2016).

4.6.2 Ferric Reducing Antioxidant Power (FRAP)

FRAP is based on reduction of TPTZ (2,4,6-tripyridyl-s-triazine) by the $FeCl_3$ $_6H_2O$, thus marking ferric reducing power of the samples. It was evident from FRAP assay results that KSM exhibited significantly ($p<0.05$) highest antioxidant power (1108.67 μM/g) followed by BSM (325.00 μM/g), while lowest values were recorded in FSM (268.33 μM/g) and PSM (259.67 μM/g) showing no statistical significance ($p>0.05$). The FRAP value of KSM was approximately 4.13 times higher than FSM and 4.27 times than PSM. Similar results were observed in previous study wherein FRAP assay values were highest in kodo bran compared to proso, foxtail and barnyard millet brans (Bijalwan *et al.* 2016).

4.6.3 TBARS (Thiobarbituric Acid Reactive Substances) assay

Another important and widely accepted protocol of antioxidant is TBARS, where thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) formed during lipid peroxidation. Thus, lower production of MDA indicates decreased lipid peroxidation which could be associated to better antioxidant potential (Zeb and Ullah, 2016). In the present study TBARS assay showed that lipid peroxidation induced oxidative stress was also lowest in KSM (0.12 µM/g) and highest in PSM (0.87 µM/g), whereas FSM (0.23 µM/g) and BSM (0.28 µM/g) showed no significant difference. This indicated that less MDA was produced in KSM compared to other bran samples. This reduced MDA levels could also be attributed with higher phytic acid content of KSM which might have decreased rate of lipid peroxidation (Graf, 1990 and Abdulwaliyu *et al.* 2019).

4.6.4 Total phenols (TP) and flavonoids

It was clear from the results that KSM had highest TP (449.27 mg GAE/100 g) and flavonoid (22.37 µg RE/ g) content, followed by BSM (197.03 mg GAE/100 g, 4.17 µg RE/ g), FSM (182.67 mg GAE/100 g, 3.51µg RE/g) and PSM (145.57 mg GAE/100 g, 4.06 µg RE/g). No significant difference was observed among BSM, FSM and PSM in TP and flavonoid content. The present study results are in agreement with previous reports where kodo millet hulls showed 3 times more TP content compared to pearl millet hulls (Chandrasekara and Shahidi, 2011). Phenolic compounds like caffeic acid, p-Coumaric acid, ferulic acid and total phenolic acid were highest in hydroxycinnamic acid bound arabinoxylans (HCA-AXs) extracts of kodo millet bran than finger, proso, barnyard and foxtail millet bran (Bijalwan *et al.* 2016).

4.6.5 Phytic Acid (PA)

Phytic acid analysis revealed that KSM (630 mg/100 g) and BSM (246.25 mg/100 g) had highest and lowest value respectively, but no significant difference was observed in phytic acid content of FSM – PSM (540.00, 516.25 mg/100 g). Similar results for many other cereal brans demonstrated phytic acid presence (Sridevi *et al.* 2011; Kundgol *et al.* 2013; Suma and Urooj, 2014; Nazni and Karuna, 2016 and Rafe *et al.* 2017). These phytic acids are known to chelate minerals reducing their bioavailability, but on the other hand phytic acids can be associated with reducing the risk of colon and breast cancer (Graf, 1990; Suma and Urooj, 2014; Nazni and Karuna, 2016 and Abdulwaliyu *et al.* 2019).

It was observed from the overall results of the present study that KSM had significantly highest ($p<0.05$) amount of TP (449.27 mg GAE /100 g), flavonoid (22.37 µg RE/g) and phytic acid (630 mg /100 g) and also demonstrated significantly highest antioxidant capacity in terms of DPPH scavenging potential, FRAP and TBARS compared to other brans. The results also reflected that antioxidant capacity was good in all the bran samples and it was higher when brans had high TP, flavonoid, phytic acid content.

Various researchers have established that cereals brans are concentrated source of phytates, phenols, flavonoids and tannins, thus, making them natural and promising source of antioxidants (Krings *et al.* 2000; Esposito *et al.* 2005 and Suma and Urooj, 2014). Even the present study demonstrated and confirmed the same that antioxidant capacity estimated via., DPPH (%) and FRAP (µM/g) was higher in brans having higher TP and flavonoids. Antioxidant capacity, TP and flavonoids followed similar trend indicating that increase in phenols and flavonoids increased antioxidant capacity of bran. These antioxidants can further help prevent risks of various degenerative diseases caused by oxidative stress (Krings *et al.* 2000; Esposito *et al.* 2005 and Suma and Urooj, 2014).

Table 4.9. Antioxidant and phytonutrient content of minor millet brans

Bran	**DPPH (%)**	**FRAP (µM/g)**	**TBARS (µM/g)**	**Flavonoid (µg RE/g)**	**TP (mg GAE /100 g)**	**Phytic acid (mg /100 g)**
FSM	14.06^{ab}	268.33^{a}	0.23^{ab}	3.51^{a}	182.67^{a}	540.00^{b}
BSM	15.26^{b}	325.00^{b}	0.28^{b}	4.17^{a}	197.03^{a}	246.25^{a}
PSM	12.94^{a}	259.67^{a}	0.87^{c}	4.06^{a}	145.57^{a}	516.25^{b}
KSM	31.13^{c}	1108.67^{c}	0.12^{a}	22.37^{b}	449.27^{b}	630.00^{c}
SEm ±	0.49	3.96	0.04	0.22	16.68	11.28

Note: Mean within same column having different alphabet show statistically significant difference at 5%. FSM: Foxtail millet bran, KSM: Kodo millet bran, PSM: Proso millet bran, BSM: Barnyard millet bran

4.7 Molar ratio of phytate and minerals of minor millet brans

As discussed earlier the phytic acid content was highest (Table 4.9) in kodo bran (630 mg/100 g), with no significant difference between PSM (516.25 mg/100 g) and FSM (540 mg/100 g), and relatively lowest content in BSM (246.25 mg/100 g). Presence of phytate content hinders minerals availability by binding with them, reducing absorption and bioavailability. Thus, even if brans' mineral content is higher, all of that may not be available due to presence of phytate. To help predict and estimate the hindering effects of phytate on mineral bioavailability, one simplest measure designed is calculating molar ratios. But to determine actual mineral bioavailability and effect of phytates on it, various *in vitro* and *in vivo* methods need to be used.

Molar ratios can be calculated for iron, zinc and calcium, as phytate binds with these minerals. Research has suggested critical values for phytate/iron (>1), phytate/zinc (>15), phytate/calcium (> 0.24) and phytate × calcium/zinc (>200) indicating inhibitory effects of phytate on minerals (ME and Ain, 2009). Molar ratios were calculated in the present study and represented in Table 4.10. It was observed that molar ratios of phytate/iron were less than the critical value in PSM (0.72) and FSM (0.70), whereas KSM (2.61) and BSM (2.35) had values above 1. This indicates less hindering effect of phytate on iron content of PSM, FSM and more effect on KSM and BSM. This can also be due to higher iron content in FSM and PSM compared to KSM and BSM.

Similarly, for phytate/zinc molar ratio all brans had values below the critical value (15) except KSM depicting that its zinc bioavailability was affected by phytate. Amongst all brans KSM had lowest zinc and highest phytic acid values that might have resulted in phytate/zinc molar ratio above critical limit for KSM. The phytate/calcium molar ratio was within the critical limit only for BSM (0.24) rest all brans had higher values. There is an uncertainty in understanding the effect of phytate on calcium and exact relation is still unknown (Gibson *et al.* 2010 and FAO/IZiNCG, 2018) as various factors are believed to hamper the absorption of calcium. It is also suggested that high amount of dietary calcium can affect absorption of zinc. Thus phytate × calcium/zinc ratio is calculated and various studies suggest the critical point above 200 (Bindra *et al.* 1986; Kwun and Kwon, 2000 and Ma *et al.* 2005). All the brans sample in present study had values below 200 for phytate × calcium/zinc and it ranged from 8.65–55.69.

Considering the molar ratio results from the present study, it can be said that FSM and PSM can be preferred where products rich in iron and zinc are needed. Similarly, BSM can be selected where zinc and calcium rich products are desired. Further to understand the effect of phytate on minerals and its bioavailability, *in vitro* and *in vivo* studies are needed.

Table 4.10. Molar ratio of phytate and minerals of minor millet brans

Bran	Phytate / Iron	Phytate / Zinc	Phytate / Calcium	Phytate × Calcium / Zinc
FSM	0.70	11.35	0.35	26.80
BSM	2.35	6.38	0.24	9.91
PSM	0.72	9.16	0.83	8.65
KSM	2.61	29.33	0.50	55.69
Critical range	>1*	>15*	>0.24*	>200*
Reference*	Ma *et al.* (2005)			

Note: FSM: Foxtail millet bran, KSM: Kodo millet bran, PSM: Proso millet bran, BSM: Barnyard millet bran

Various processes can be employed to reduce or decrease the phytate content and improve bioavailability of minerals. These processes can be tedious as phytate is relatively heat stable and not easily degraded. Food processing methods such as extrusion cooking or processes where higher temperatures are utilised, fermentation, soaking, and phytase enzymes can be used to reduce the phytate content (Ma *et al.* 2005; Schlemmer *et al.* 2009; Gupta *et al.* 2015 and FAO/IZiNCG, 2018).

4.8 Physical and functional properties minor millet brans blended flours and their products

To develop designer buns and muffins, refined wheat flour was blended with minor millet brans viz., foxtail, barnyard, proso and kodo bran each at 10, 15, 20, 25 and 30 per cent. Refined wheat flour without bran addition was considered as control. These flours were assessed for their physicochemical and functional properties like water absorption, water solubility index, oil absorption and foaming capacity.

4.8.1 Flour blended with minor millet brans

The results for water absorption, water solubility index, oil absorption and foaming capacity are presented in Table 4.11.

4.8.1.1 Water absorption capacity (WAC) and Water solubility index (WSI)

It was observed that refined wheat flour treated as control (RWF) had lowest water absorption capacity (209.20 g/100 g) compared to bran incorporated flours. Only foxtail bran incorporated at 10 per cent (FSM 10) was comparable to RWF. With increase in bran per cent there was an increase in water absorption capacity of the flours. All flours blended with 30 per cent bran viz., FSM30 (245.74 g/100 g), BSM30 (245.79 g/100 g), PSM30 (248.42 g/100 g) and KSM30 (248.18 g/100 g) exhibited no significant difference

($p<0.05$) in WAC. However, KSM25 (247.56 g/100 g) and PSM25 (246.51 g/100g) were not significantly different from other flours blended with 30 per cent bran. PSM10, PSM15, KSM15, FSM20 did not differ statistically and had similar WAC.

It was noted from the results that all brans enhanced the water absorption capacity. A direct relation was observed where, an increase in bran percentage resulted in increased water absorption capacity. This may be associated with the fiber present in the bran containing polysaccharides which leads to increased water absorption by forming more hydroxyl groups and increasing the water hydrogen bonding (Chaplin, 2003, Rafe *et al.* 2017 and Lauková *et al*. 2019). Other factors like protein, fat content and particle size also influence the water absorption (Sairam *et al.* 2011 and Dhankhar *et al.* 2019). Thus, these differences in WAC amongst brans can be attributed to differences in components like soluble, insoluble fractions of bran. This understanding of WAC will help the food processing industry select brans to be used in various formulations. All bran blended flours in the present study exhibited higher WAC than RWF indicating that, they can be used in product development, where moisture retention is required in final products. In bakery products this can help to maintain freshness of product (Sairam *et al.* 2011). These results are similar to those studies that reported an increase in water absorption after bran addition (Bagheri and Seyedein, 2011; El-Sharnouby *et al.* 2012 and Egbedike *et al.* 2016).

A decreasing trend was observed in WSI of bran incorporated flours. RWF had the highest water solubility and among bran incorporated flours, BSM10, BSM15, BSM 20, KSM10, KSM15, KSM20 showed similarity with RWF. Water solubility significantly decreased in flours blended with 30 per cent foxtail (FSM30), proso (PSM30) and kodo (KSM30) bran compared to control. Irrespective of bran type, as the bran incorporation increased the water solubility index decreased. Similar results were demonstrated by Pauline *et al*. (2020).

4.8.1.2 Oil absorption capacity (OAC)

The oil absorption capacity of flour helps to retain flavour, imparts better mouthfeel to products and is desired in cake batters (Ahmad *et al.* 2015 and Rafe *et al.* 2017). The oil absorption capacity of control flour (RWF) and all flours blended with bran had no significant difference. Still, there was slight reduction in bran enriched flour's OAC compared to that of refined wheat flour (RWF). This slight reduction might be associated with the full fat brans used in present study which were not defatted. Previous studies indicate that presence of fat in flours can reduce oil absorption (Chandra *et al.* 2015). Factors like variability in protein content and its hydrophobic and hydrophilic amino acids also influence oil absorption capacity (Dhankhar *et al.* 2019).

Table 4.11. Functional properties of flour blended with minor millet brans

Flour	WAC (g/100g)	WSI (%)	OAC (g/100g)	FC (%)
RWF	209.20^{a}	7.41^{i}	246.70	19.33^{k}
FSM 10	217.31^{ab}	6.30^{bcde}	227.76	16.00^{ghij}
FSM 15	232.89^{efg}	6.25^{cde}	225.09	12.67^{abcdef}
FSM 20	230.73^{def}	6.24^{cde}	223.19	11.70^{abcd}
FSM 25	231.43^{ef}	5.94^{abc}	221.68	11.27^{ab}
FSM 30	245.74^{h}	5.54^{a}	220.32	10.26^{a}
BSM 10	219.86^{bc}	7.38^{i}	236.18	18.67^{jk}
BSM 15	226.25^{cde}	7.39^{i}	234.42	16.67^{hijk}
BSM 20	231.52^{ef}	7.31^{i}	233.88	14.67^{efghi}
BSM 25	235.79^{fg}	7.14^{ghi}	233.50	12.00^{abcde}
BSM 30	245.79^{h}	7.11^{ghi}	233.55	10.67^{ab}
PSM 10	228.76^{def}	6.64^{defg}	230.39	16.80^{ijk}
PSM 15	231.13^{def}	6.39^{cde}	227.64	14.00^{cdefgh}
PSM 20	240.52^{gh}	6.29^{bcde}	224.57	14.80^{fghi}
PSM 25	246.51^{h}	5.87^{ab}	222.11	14.33^{defghi}
PSM 30	248.42^{h}	5.69^{a}	220.12	12.80^{abcdef}
KSM 10	222.94^{bcd}	7.36^{i}	237.42	16.00^{ghij}
KSM 15	228.29^{def}	7.27^{hi}	234.85	14.67^{efghi}
KSM 20	235.70^{fg}	6.91^{fghi}	234.40	13.33^{bcdefg}
KSM 25	247.56^{h}	6.79^{efgh}	233.95	12.00^{abcde}
KSM 30	248.18^{h}	6.56^{def}	233.01	11.33^{abc}
SEm ±	2.89	0.18	6.17	0.96

Note: Means ± SEm represented within same column having different alphabet show statistically significant difference at 5% and no alphabet indicates no significant difference.

4.8.1.3 Foaming capacity (FC)

Foaming capacity also showed a decrease with addition of bran (Table 4.11). In the present study, RWF had highest FC and the bran blended flours had significantly ($p < 0.05$) lowest foaming capacity. The FC significantly reduced from 19.33 per cent in

RWF to 10.26 per cent (FSM30), 10.67 per cent (BSM30), 12.80 per cent (PSM30) and 11.33 per cent (KSM30) as incorporation reached 30 per cent irrespective of bran type. FC mainly depends on protein, its flexibility, elasticity, cohesiveness, denaturation etc. (Chandra *et al.* 2015 and Dhankhar *et al.* 2019). The present study results are in accordance with the study of Egbedike *et al.* (2016) wherein addition of rice bran decreased foaming capacity, suggesting that protein in bran is globular and not easily denatured. A higher foaming capacity was observed for rice brans in another study, associated to pH changes and varietal differences (Rafe *et al.* 2017). The increase in bran percentage led to an increase in WAC, whereas decreased WSI and FC while there was no significant effect on OAC.

4.8.2 Minor millet bran incorporated products

Designer buns and muffins were developed using all bran blended with flour at 0, 10, 15, 20, 25 and 30 per cent. Both buns and muffins were baked at 180 °C for 22 minutes as the previous literature suggested that best texture and phytonutrients were retained in muffins when baked at 180 °C (Mildner-Szkudlarz *et al.* 2016).

4.8.2.1 Physical characteristics of muffins

Results of one-way ANOVA for weight and height showed that there was no significant difference ($p>0.05$) within any treatment amongst all bran blended muffins (Table 4.12). In all baked muffins weight reduced and height increased compared to raw muffins. This reduction in baked weight compared to raw weight is due to loss of moisture during baking. The increase in height after baking was due to the rise created while air bubbles escape creating porous and spongy texture (Nazni and Karuna, 2016).

With increase in bran incorporation there was a non-significant increase in weight and decrease in height for all baked muffins. A reduction in bran blended muffins' height can be due to the reduced air bubbles escaping during baking. This decrease in height can also be due to the fiber in bran that disrupts the gluten network. Similarly, a previous study on muffins enriched with dietary fibre obtained from kimchi showed increase in weight and decrease in height as dietary fiber's addition increased from 0 to 4 per cent (Heo *et al.* 2019). Also, another study showed that hardness of muffins increased as barnyard bran incorporation increased from 0 to 30 per cent and hardness was attributed to lower number of air bubbles and denser matrix (Nazni and Karuna, 2016).

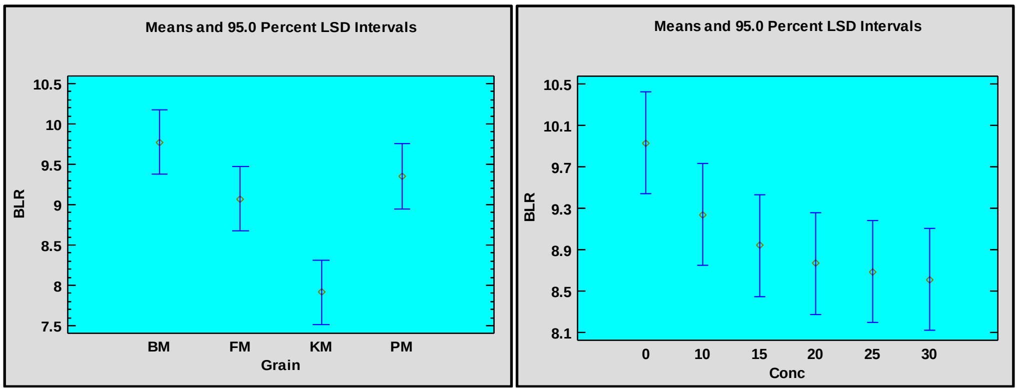
Table 4.12. Physical characteristics of kodo, proso, foxtail and barnyard muffins

	Treatment		M0	M10	M15	M20	M25	M30	Gr. M	Sem ±
Kodo millet	**Weight (g)**	**BB**	32.80	33.00	32.90	33.30	33.10	33.10	33.03	0.23
		AB	30.30	30.60	30.50	30.50	30.40	30.40	30.45	0.49
	Height (cm)	**BB**	1.68	1.60	1.66	1.64	1.54	1.64	1.63	0.05
		AB	3.72	3.72	3.70	3.70	3.68	3.68	3.70	0.11
	BLR (%)		8.18	8.14	7.86	7.80	7.78	7.75	7.92	0.55
Proso millet	**Weight (g)**	**BB**	33.60	33.30	33.40	33.80	33.60	33.50	33.53	0.24
		AB	30.20	30.30	30.40	30.70	30.70	30.60	30.48	0.63
	Height (cm)	**BB**	1.68	1.62	1.64	1.68	1.66	1.68	1.66	0.05
		AB	3.86	3.82	3.86	3.86	3.84	3.84	3.85	0.08
	BLR (%)		10.80	9.91	9.26	8.89	8.64	8.61	9.35	0.58
Foxtail millet	**Weight (g)**	**BB**	33.20	33.10	33.10	33.40	33.20	33.10	33.18	0.19
		AB	29.60	30.10	30.00	30.40	30.40	30.30	30.13	0.38
	Height (cm)	**BB**	1.58	1.56	1.56	1.56	1.54	1.58	1.56	0.03
		AB	3.46	3.46	3.34	3.34	3.38	3.44	3.40	0.12
	BLR (%)		9.34	9.34	9.06	8.95	8.99	8.76	9.07	0.93
Barnyard millet	**Weight (g)**	**BB**	33.30	33.40	33.40	33.12	33.10	33.20	33.25	0.17
		AB	29.50	30.20	30.20	30.00	30.00	30.10	30.00	0.23
	Height (cm)	**BB**	1.60	1.59	1.62	1.62	1.60	1.62	1.61	0.05
		AB	3.32	3.28	3.28	3.28	3.26	3.24	3.28	0.10
	BLR (%)		11.41	9.56	9.58	9.42	9.35	9.33	9.77	0.82

Note: M: muffin; 0,10,15,20,25,30: per cent bran incorporation. BB: Before baking, AB: after baking, BLR: Baking Loss Rate. Gr. M: Grand mean.

Lesser baking loss rate (%) was observed in bran blended muffins compared to control, however there was no significant difference (p>0.05). The BLR (%) decreased with increasing bran percentage. In kodo bran muffin the BLR decreased from 8.18 per cent in control (KM0) to 7.75 per cent (KM30) in 30 per cent bran enriched muffin (Table 4.12). Similarly decrease in proso, barnyard and foxtail bran muffins BLR decreased from 10.80 (PM0), 11.41 (BM0) and 9.34 (FM0) to 8.61 (PM30), 9.33 (BM30) and 8.76 (FM30) respectively (Table 4.12). Factorial analysis was further conducted to understand the effect of grain and per cent bran addition on BLR. It was observed that BLR was significantly lower in kodo bran enriched muffins irrespective of the per cent bran incorporation. No significant difference (p>0.05) was observed among BLR of foxtail, proso and barnyard bran blended muffins. As the bran concentration increased from 0 to

30 per cent BLR decreased (Fig 4.9). BLR in control (0 %) muffins was significantly higher than muffins with 15–30 per cent bran. No significant difference was observed between BLR of control and 10 per cent bran blended muffins (Fig 4.9). This decrease in BLR indicated that bran addition increased final weight of product and it can be attributed to water absorption capacity of bran that negatively affected the gluten networks, volume and height of muffins (Heo *et al.* 2019).



(a) (b)

Fig 4.9 Effect of grain (a) and bran per cent (b) on baking loss rate of muffins

Note: Values expressed as Mean ± SE. BLR: Baking Loss Rate. In the figure grain indicates respective grain from which bran was obtained. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Bran per cent is bran incorporation at 0, 10, 15, 20, 25 and 30%.

4.8.2.2 Physical characteristics of buns

Results for physical characteristics of buns are presented in Table 4.13. From the results it was observed that there was a non-significant increase in weight of baked bran blended buns. Another study also recorded a rise in bun weight after bran incorporation (Kaur *et al.* 2017).

Bran incorporation significantly ($p<0.05$) reduced the height of baked buns compared to control. The height of baked buns was highest in control *i.e.*, BB0 (4.54 cm), KB0 (4.64 cm), FB0 (4.70 cm) and PB0 (4.58 cm) which significantly reduced in 30 per cent bran incorporated buns *i.e.*, BB30 (3.66 cm), KB30 (3.74 cm), FB30 (4.20 cm) and PB30 (3.94 cm) respectively. However, bran addition up to 10 per cent was on par with control for kodo, foxtail and barnyard, except proso bran bun. In proso all bran blended buns were significantly different from control (PB0). In barnyard and kodo bran blended buns there was no significant difference ($p>0.05$) between 10 per cent and 15 per cent while significant difference was noted among 20 per cent, 25 per cent and 30 per cent. In foxtail bran buns, despite the decrease in height, FB10 and FB15 were on par with control (FB0) indicating that bran addition up to 15 per cent did not affect the height significantly ($p>0.05$). Further FB20 was on par with FB10 and FB15, but different from FB0, while no significant difference between FB25 and FB30. In proso bun there was no significant difference among PB20, PB25 and PB30.

Baking loss rate (%) showed that buns with all brans had lower values compared to their respective controls however, the reduction was not significant ($p>0.05$). Baking loss rate (%) ranged from 6.49 (KB0), 8.96 (PB0), 6.90 (FB0), 7.70 (BB0) to 5.82 (KB30), 7.30 (PB30), 6.01 (FB30) and 6.79 (BB30) in kodo, proso, foxtail and barnyard bran buns respectively (Table 4.13).

Table 4.13. Physical characteristics of kodo, proso, foxtail and barnyard buns

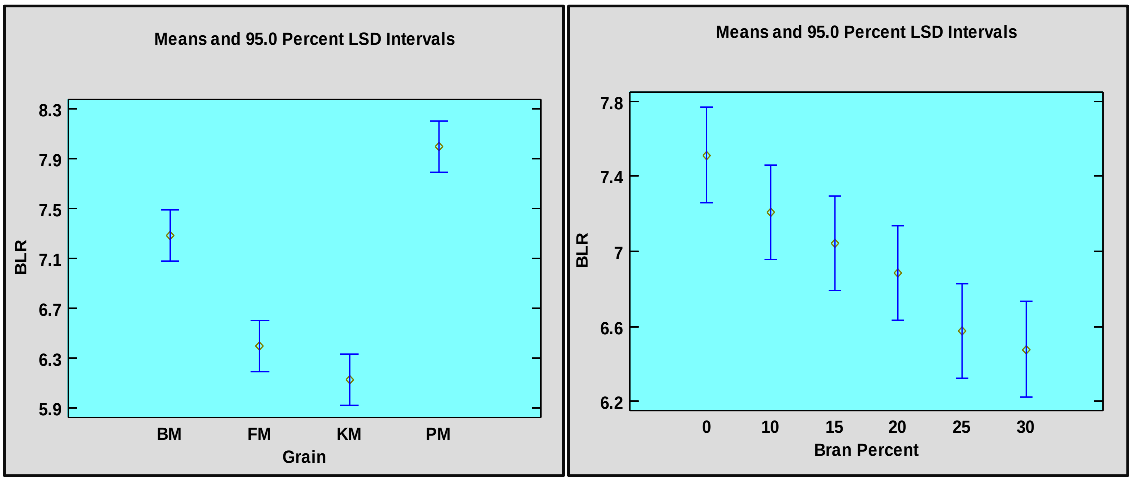
	Treatment		B0	B10	B15	B20	B25	B30	Gr. M	Sem ±
Kodo millet	**Weight (g)**	**BB**	86.20	86.60	86.90	87.30	87.90	87.50	87.07	0.43
		AB	82.20	82.10	82.20	82.40	82.40	82.40	82.28	0.64
	Height (cm)	**BB**	2.88	2.88	2.84	2.88	2.82	2.84	2.86	0.05
		AB	4.64^{e}	4.50^{de}	4.44^{cd}	4.28^{c}	3.96^{b}	3.74^{a}	4.26	0.06
	BLR (%)		6.49	6.36	6.09	6.07	5.91	5.82	6.12	0.44
Proso millet	**Weight (g)**	**BB**	87.70	87.90	87.50	87.70	87.50	87.40	87.62	0.36
		AB	82.50	83.50	83.50	83.50	83.40	83.80	83.37	0.32
	Height (cm)	**BB**	2.82	2.78	2.74	2.78	2.76	2.78	2.78	0.06
		AB	4.58^{c}	4.42^{b}	4.38^{b}	4.06^{a}	4.02^{a}	3.94^{a}	4.23	0.05
	BLR (%)		8.96	8.30	8.10	7.87	7.42	7.30	7.99	0.38
Foxtail millet	**Weight (g)**	**BB**	88.20	88.10	88.00	88.20	88.00	88.10	88.10	0.47
		AB	82.80	83.60	83.70	83.80	83.90	84.10	83.65	0.29
	Height (cm)	**BB**	2.84	2.88	2.84	2.86	2.84	2.86	2.85	0.08
		AB	4.70^{c}	4.64^{bc}	4.52^{bc}	4.44^{b}	4.16^{a}	4.12^{a}	4.43	0.09
	BLR (%)		6.90	6.58	6.47	6.31	6.13	6.01	6.40	0.37
Barnyard millet	**Weight (g)**	**BB**	88.00	88.00	87.70	87.70	88.20	88.40	88.00	1.03
		AB	82.00	82.10	82.10	82.20	82.02	82.40	82.17	0.72
	Height (cm)	**BB**	2.94	2.84	2.82	2.82	2.84	2.84	2.85	0.07
		AB	4.54^{e}	4.40^{de}	4.36^{cd}	4.20^{c}	3.86^{b}	3.66^{a}	4.17	0.06
	BLR (%)		7.70	7.60	7.52	7.29	6.83	6.79	7.29	0.32

Note: B: bun; 0,10,15,20,25,30: per cent bran incorporation. BB: Before baking, AB: after baking, BLR: Baking Loss Rate. Gr. M: Grand mean. Means represented within same row having different alphabet show statistically significant difference at 5% and means with no alphabet indicates no significant difference within the treatments.

Further, effect of each grain (from where bran was obtained) and bran per cent (0,10, 15, 20, 25 and 30 %) was observed by analyzing the data using factorial design

(Fig 4.10). It was observed from the results that irrespective of bran concentration BLR was lowest in kodo and foxtail millet with no significant difference ($p>0.05$), followed by barnyard and proso having a significant difference ($p<0.05$). When compared amongst the treatments, decrease in BLR was observed as per cent bran increased (Fig 4.10). Amongst bran concentrations BLR for 10 and 15 per cent bran blended bun was on par with control (0 % bran) bun and BLR for 30 per cent were similar to 20 and 25 per cent. Buns with 20, 25 and 30 per cent bran recorded significantly lower BLR values than control (Fig 4.10).

This decrease in height and baking loss rate can be attribute to disruption of gluten networks due to bran presence in flours. A decrease in height also indicated that the bran incorporated buns were not as spongy as control. Reduction in baking loss rate, height and volume due to negative effect of bran on gluten was also reported in previous studies (Bagheri and Seyedein, 2011 and Kaur *et al.* 2017).



(a) (b)

Fig 4.10 Effect of grain (a) and bran per cent (b) on baking loss rate of buns

Note: Values expressed as Mean ± SE. BLR: Baking Loss Rate. In the figure grain indicates respective grain from which bran was obtained. FM- Foxtail millet bran, KM- kodo millet bran, PM- Proso millet bran, BM- Barnyard millet bran. Bran per cent is bran incorporation at 0, 10, 15, 20, 25 and 30%.

4.8.3 Sensory evaluation of designer muffins and buns

Sensory evaluation was conducted for muffins and buns prepared from kodo, proso, foxtail and barnyard millet bran at various incorporation levels (0 %, 10 %, 15 %, 20 %, 25 % and 30 %) and the results are presented in Table 4.14 – 4.23.

It can be observed from the tables (Table 4.14 – 4.17) that incorporation of bran up to 30 per cent non-significantly reduced the scores for all parameters (appearance, texture, flavor, taste and overall acceptability) except for barnyard bran blended muffins. Reduced sensory scores with increased bran incorporation in the present study was due to husky taste, and grainy texture imparted by addition of bran. Similar results were demonstrated by Lebesi and Tzia (2011). The colour also darkened slightly with increasing per cent of bran addition compared to control resulting in reduction of scores. Similarly, previous studies also provided evidence that addition of bran darkens the colour of products (Gajula *et al.* 2008 and Sharif *et al.* 2009).

The overall acceptability scores for kodo, foxtail, proso and barnyard muffins ranged from 7.71 – 7.33, 7.71 – 7.95, 7.71 – 7.10 and 8.33 – 7.14 respectively (Table 4.14 – 4.17). All the scores were above 7 making it evident that 30 % bran blended muffins were acceptable and liked moderately by the panelists. Overall acceptability scores for barnyard bran muffins showed that (Table 4.17) as bran percentage increased scores decreased significantly ($p<0.05$) compared to control. However, there was no significant difference between BM10, BM15 and BM20. Although significantly different from BM10 and BM15, BM25 and BM30 were on par with BM20.

The present study results show that addition of bran up to 30 per cent in refined wheat flour in muffins was acceptable. In contrast, previous studies report only 8 – 10 per cent rice, wheat and oat bran (Lebesi and Tzia, 2011 and Romjaun and Prakash, 2013) and up to 15 per cent barnyard millet bran Nazni and Karuna (2016) being acceptable which is much lower compared to the present study.

Based on the present study, highest level of bran incorporation *i.e.*, 30 per cent in all muffins (kodo, foxtail, proso, and barnyard bran) was used for further study. All bran blended muffins are presented in Plate 4.1.

Table 4.14. Mean sensory scores for muffins prepared using kodo millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
KM0	7.81	8.10^{b}	7.71	7.52	7.52	7.71
KM10	7.62	7.57^{ab}	7.33	7.43	7.62	7.57
KM15	7.86	8.10^{b}	7.48	7.52	7.71	7.81
KM20	7.71	7.67^{ab}	7.38	7.43	7.48	7.57
KM25	7.71	7.67^{ab}	7.38	7.24	7.62	7.43
KM30	7.71	7.38^{a}	7.33	7.05	7.33	7.33
SEm ±	0.20	0.21	0.25	0.25	0.27	0.23

Note: KM: kodo bran muffin; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.15. Mean sensory scores for muffins prepared using foxtail millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
FM0	8.00	8.10	7.62	8.00	7.71	7.71
FM10	7.81	7.86	7.48	7.57	7.71	7.67
FM15	7.33	7.62	7.43	7.62	7.62	7.29
FM20	7.71	7.76	7.24	7.48	7.62	7.52
FM25	7.67	7.62	7.62	7.33	7.24	7.24
FM30	8.00	7.81	7.90	7.90	7.90	7.95
SEm ±	0.18	0.19	0.27	0.24	0.23	0.24

Note: FM: foxtail bran muffin; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.16. Mean sensory scores for muffins prepared using proso millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
PM0	7.95	7.86	7.95	7.29	7.67	7.71
PM10	7.86	7.29	7.52	7.00	7.62	7.62
PM15	7.81	7.67	7.67	7.24	7.67	7.62
PM20	7.52	7.48	7.52	7.38	7.71	7.71
PM25	7.24	7.19	7.10	7.00	7.19	7.19
PM30	7.48	7.14	7.24	7.33	7.33	7.10
SEm ±	0.21	0.21	0.22	0.33	0.26	0.25

Note: PM: Proso bran muffin; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.17. Mean sensory scores for muffins prepared using barnyard millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
BM0	8.38^{d}	8.24^{c}	7.95^{b}	8.10^{b}	8.24^{b}	8.33^{c}
BM10	7.95^{cd}	7.90^{bc}	7.33^{a}	7.52^{ab}	7.62^{a}	7.71^{b}
BM15	7.90^{bcd}	7.67^{abc}	7.52^{ab}	7.52^{ab}	7.57^{a}	7.71^{b}
BM20	7.43^{abc}	7.48^{ab}	7.38^{ab}	7.33^{a}	7.29^{a}	7.33^{ab}
BM25	7.38^{ab}	7.14^{a}	7.14^{a}	7.14^{a}	7.14^{a}	7.10^{a}
BM30	7.29^{a}	7.14^{a}	7.29^{a}	7.05^{a}	7.19^{a}	7.14^{a}
SEm ±	0.19	0.22	0.22	0.22	0.20	0.17

Note: BM: barnyard bran muffin; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Further to select one final bran, all 30 per cent bran blended muffins from each bran viz., kodo, proso, foxtail and barnyard along with control (without bran) were subjected for sensory evaluation. The sensory evaluation results of muffins for CM (control), KM30, PM30, FM30 and BM30 are documented in Table 4.18. It was found that CM scored a higher score than all bran incorporated muffins. In terms of appearance CM scored highest (8.43) followed by FM30 (7.86), PM30 (7.52), BM30 (7.43) and KM30 (7.24). FM30 was on par with CM and significantly different from KM30. Flavor, taste and overall acceptability of bran incorporated muffins exhibited a significant difference from control. However, there was no significant difference amongst the bran incorporated muffins.

As all the bran blended muffins scored 7 or above for overall acceptability, nutritional, antioxidant and phytonutrient content were considered in order to select final bran muffin. The highest antioxidant and phytonutrient content kodo bran muffin (KM30) was chosen for further analysis and supplementation study. All 30 per cent bran blended and control muffins are presented in Plate 4.2.

Plate 4.1 Muffins prepared from kodo (KM), proso (PM), foxtail (FM) and barnyard (BM) bran at different incorporation levels (0, 10, 15, 20, 25 and 30%)

Table 4.18. Mean sensory scores for 30 per cent bran blended muffins

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
KM30	7.24^{a}	7.33^{ab}	7.19^{a}	7.24^{a}	7.00^{a}	7.00^{a}
PM30	7.52^{ab}	7.81^{bc}	7.76^{ab}	7.57^{a}	7.52^{a}	7.57^{a}
FM30	7.86^{bc}	7.33^{ab}	7.14^{a}	7.52^{a}	7.43^{a}	7.48^{a}
BM30	7.43^{ab}	7.14^{a}	7.52^{a}	7.33^{a}	7.33^{a}	7.48^{a}
CM	8.43^{c}	8.24^{c}	8.29^{b}	8.29^{b}	8.33^{b}	8.48^{b}
SEm ±	0.21	0.17	0.22	0.20	0.22	0.21

Note: CM control, FM30 foxtail bran at 30%, KM30 kodo bran at 30%, PM30 proso bran at 30% and BM30 barnyard bran at 30%. Means represented within same column having different alphabet show statistically significant difference at 5 %. and means with no alphabet indicates no significant difference.

Plate 4.2 Muffins prepared with 30 per cent bran incorporation

Note: CM control, FM30 foxtail bran at 30%, KM30 kodo bran at 30%, PM30 proso bran at 30% and BM30 barnyard bran at 30%.

Results of sensory evaluation of the buns are presented in Table 4.19 – 4.22 and all bran enriched buns are depicted in Plate 4.3. In bran incorporated buns the sensory scores decreased significantly ($p<0.05$) for all parameters (appearance, colour, flavour, texture, taste and overall acceptability) compared to their respective controls. Foxtail, proso and barnyard buns with 20 per cent bran scored above 7 for overall acceptability showing that they were liked moderately, while buns with 20 per cent kodo bran scored below 7 indicating that they were liked slightly by the semi-trained panel members. Amongst all brans, bun with 25 per cent and 30 per cent bran scored less than 7. The reasons for reduction of sensory scores can be attributed to the sandy taste, rough texture imparted by addition of bran. Not only the taste and texture, but colour was also affected due to addition of bran. The colour of buns became darker as the bran per cent increased, compared to control. Similarly, previous studies also provided abundance of evidence that addition of bran darkens the colour of breads and buns (Dahlberg *et al.* 2004; Hines, 2007; Kaur *et al.* 2017; Hussein and Ibrahim, 2019 and Ndlala *et al.* 2019).

For foxtail millet bran buns (Table 4.19) FB30 recorded the lowest score for overall acceptability (6.67) compared to control (8.14). Trend for overall acceptability was seen with control having highest scores *i.e.,* FB0 (8.14) followed by FB10 (7.52, FB15 (7.19), FB20 (7.14), FB25 (6.71) and FB30 (6.67). Amongst the buns that scored above 7, FB15 and FB20 had no significant difference thus, buns with higher bran content *i.e.,* FB20 was selected for further sensory evaluation.

Sensory evaluation results for kodo bran (Table 4.19) revealed that control with no bran (KB0) scored highest for overall acceptability followed by KB10, KB20, KB15, KB25 and KB30. When bran percentage increased above 10 per cent the acceptability was decreased significantly ($p<0.05$). The overall acceptability scores for KB15, KB20 and KB25 showed no significant difference and all the scores were below 7, but KB20 had comparatively higher scores (6.81) thus was selected for further study.

In proso bran bun (Table 4.20) there was no significant difference among PB0, PB10, PB15 and PB20 indicating that buns with 20 per cent proso bran were acceptable by the semi-trained panelist. The overall acceptability scores for buns up to 20 per cent bran addition were 7 or above, whereas it was below 7 for PB25 and PB30. The sensory scored of barnyard millet bran buns were BB0 > BB10 >BB15 > BB20 > BB25 > BB30 (Table 4.21). The overall acceptability of BB0 and BB10 had no significant difference. Similarly, BB15 and BB20 showed no significant difference and scored above 7 for overall acceptability. Hence, BB20 muffin was selected for further studies.

Results in the present study revealed that millet bran incorporated buns up to 20 per cent were highly acceptable which is slightly higher than previous studies that reported addition of rice, wheat and oat bran in breads up to 10 – 15 per cent was highly acceptable (Bagheri and Seyedein, 2011; Saccotelli *et al.* 2017, Kaur *et al.* 2017 and Bartalné-Berceli *et al.* 2018).

Thus, it was noted that buns with 20 per cent bran were acceptable. Further these accepted buns and control were subjected to sensory evaluation for selecting one bran amongst all the four brans and results are documented in Table 4.23. It can be observed from the results that control bun (CB) had highest acceptability, followed by FB20, PB20, BB20 and KB20. Flavour and taste in all bran blended buns including control (CB) showed no significant difference ($p<0.05$). It was observed that kodo bran bun (KB20) had significantly lowest acceptability and all parameters scored less than 7 thus it was eliminated. Amongst all the buns FB20 had scores for all parameters on par with control showing no significant difference.

Considering this, FB20 was selected for further analysis and supplementation study. All 20 per cent bran blended buns are presented in Plate 4.4.

Table 4.19. Mean sensory scores for buns prepared using foxtail millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
FB0	8.19^{c}	8.10^{c}	8.14^{d}	7.90^{c}	8.00^{c}	8.14^{c}
FB10	7.57^{b}	7.48^{b}	7.52^{c}	7.14^{b}	7.33^{bc}	7.52^{bc}
FB15	7.43^{ab}	7.10^{ab}	7.19^{bc}	6.81^{ab}	6.86^{ab}	7.19^{ab}
FB20	7.29^{ab}	7.24^{b}	7.00^{abc}	7.05^{b}	7.10^{ab}	7.14^{ab}
FB25	7.24^{ab}	7.19^{b}	6.71^{ab}	6.62^{ab}	6.62^{a}	6.71^{a}
FB30	6.90^{a}	6.62^{a}	6.48^{a}	6.14^{a}	6.48^{a}	6.67^{a}
SEm ±	0.20	0.19	0.22	0.26	0.25	0.22

Note: FB: Foxtail bran bun; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.20. Mean sensory scores for buns prepared using kodo millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
KB0	8.14^{d}	7.95^{d}	8.14^{d}	7.57^{b}	7.71^{b}	8.00^{c}
KB10	7.57^{cd}	7.43^{cd}	7.57^{cd}	6.95^{ab}	7.24^{ab}	7.33^{bc}
KB15	6.86^{abc}	6.76^{bc}	6.86^{bc}	6.57^{a}	6.48^{a}	6.71^{ab}
KB20	6.90^{bc}	6.62^{b}	6.71^{b}	6.71^{a}	6.76^{a}	6.81^{ab}
KB25	6.81^{ab}	6.57^{b}	6.67^{b}	6.62^{a}	6.71^{a}	6.67^{ab}
KB30	6.14^{a}	5.48^{a}	5.90^{a}	6.43^{a}	6.48^{a}	6.19^{a}
SEm ±	0.27	0.25	0.26	0.30	0.30	0.29

Note: KB: Kodo bran bun; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.21. Mean sensory scores for buns prepared using proso millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
PB0	7.90^{b}	7.86^{c}	8.14^{c}	7.81^{c}	7.86^{d}	7.81^{c}
PB10	7.95^{b}	7.76^{bc}	7.67^{bc}	7.33^{bc}	7.67^{cd}	7.86^{c}
PB15	7.71^{ab}	7.24^{ab}	7.33^{ab}	7.00^{ab}	7.29^{bc}	7.43^{bc}
PB20	7.48^{ab}	7.10^{a}	7.24^{ab}	6.57^{a}	6.86^{ab}	7.00^{ab}
PB25	7.24^{a}	6.71^{a}	6.90^{a}	6.62^{a}	6.71^{a}	6.71^{a}
PB30	7.24^{a}	6.90^{a}	7.05^{a}	6.67^{a}	6.76^{a}	6.76^{a}
SEm ±	0.18	0.20	0.21	0.23	0.18	0.18

Note: PB: Proso bran bun; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.22. Mean sensory scores for buns prepared using barnyard millet bran

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
BB0	8.29^{d}	8.24^{d}	8.10^{d}	7.90^{c}	7.86^{c}	8.29^{d}
BB10	8.00^{cd}	7.86^{cd}	7.81^{d}	7.76^{c}	7.86^{c}	8.00^{d}
BB15	7.71^{bc}	7.57^{c}	7.67^{cd}	7.43^{bc}	7.24^{b}	7.48^{c}
BB20	7.29^{b}	7.00^{ab}	7.10^{bc}	7.05^{ab}	7.00^{ab}	7.14^{bc}
BB25	7.24^{b}	7.05^{b}	6.90^{b}	6.71^{a}	6.48^{a}	6.81^{ab}
BB30	6.48^{a}	6.52^{a}	6.33^{a}	6.48^{a}	6.57^{a}	6.57^{a}
SEm ±	0.18	0.18	0.23	0.21	0.20	0.18

Note: BB: Barnyard bran bun; 0,10,15,20,25,30: per cent bran incorporation. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Table 4.23. Mean sensory scores for 20 per cent bran blended buns

Variations	Appearance	Colour	Texture	Flavour	Taste	Overall acceptability
KB20	6.90^{a}	6.81^{a}	6.76^{a}	6.81^{NS}	6.57^{NS}	6.71^{a}
PB20	7.48^{ab}	7.29^{abc}	7.14^{ab}	6.95^{NS}	7.14^{NS}	7.29^{abc}
FB20	7.86^{bc}	7.76^{bc}	7.48^{bc}	7.43^{NS}	7.29^{NS}	7.43^{bc}
BB20	7.10^{a}	7.19^{ab}	7.33^{abc}	7.24^{NS}	7.05^{NS}	7.00^{ab}
CB	8.14^{c}	7.86^{c}	7.86^{c}	7.48^{NS}	7.48^{NS}	7.76^{c}
SEm ±	0.21	0.21	0.22	0.21	0.24	0.20

Note: CB control, FB20 foxtail bran at 20%, KB20 kodo bran at 20%, PB20 proso bran at 20% and BB20 barnyard bran at 20%. Means represented within same column having different alphabet show statistically significant difference at 5 % and means with no alphabet indicates no significant difference.

Plate 4.3 Buns prepared from kodo (KB), proso (PB), foxtail (FB) and barnyard (BB) bran at different incorporation levels (0, 10, 15, 20, 25 and 30 %)

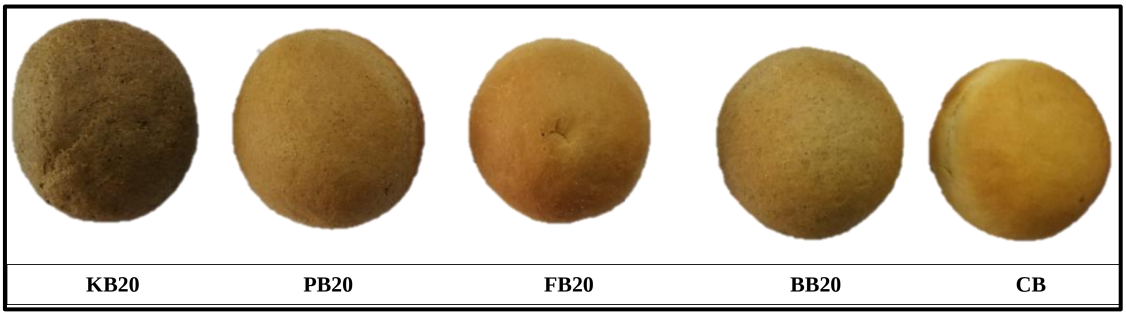


Plate 4.4 Buns prepared with 20 per cent bran incorporation

Note: CB control, FB20 foxtail bran at 20%, KB20 kodo bran at 20%, PB20 proso bran at 20% and BB20 barnyard bran at 20%.

4.9 Nutrient profile of best accepted minor millet bran incorporated designer muffins and buns

Best selected products muffin (KM30) and bun (FB20), along with the respective controls (KM0 and FB0) were analyzed for nutrient, antioxidant and phytonutrient composition before starting the supplementation studies.

4.9.1 Proximate composition

Proximate composition of muffins (KM0 and KM30) and buns (FB0 and FB20) are presented in Table 4.24. The moisture content ranged from 24.84 ± 1.271 g/100 g (FB20) to 24.95 ± 0.197 g/100 g (FB0) and 25.08 ± 1.588 g/100 g (KM0) to 25.40 ± 0.563 g/100g (KM30) in buns and muffins respectively. In both the products, protein content (g/100 g) increased from 5.65 ± 1.157 (FB0) to 6.83 ± 0.804 (FB20) in buns and from 6.96 ± 0.111 (KM0) to 7.03 ± 0.303 (KM30) in muffins with the addition of bran. The percentage increase in protein content was 20 per cent for buns while it was only 1 per cent for muffins after bran incorporation compared to their respective controls. The protein content of FB20 bun was higher than that of KM30 muffin. This difference can be attributed to foxtail bran's high protein content than kodo bran (Table 4.7). The previous investigations also confirmed that foxtail millet bran had higher amount of protein compared to kodo millet bran (Liang *et al.* 2010; Bisoi *et al.* 2012 and Sarma *et al.* 2017). Similarly, ash content also increased in both buns (from 1.63 ± 0.995 to 2.05 ± 0.048 g/100 g) and muffins (from 1.23 ± 0.031 to 1.62 ± 0.005 g/100 g) indicating increase in mineral content of products due to incorporation of bran.

TDF in buns and muffins significantly increased upon the incorporation of bran from their controls. It increased from 0.74 ± 0.009 (FB0) to 2.73 ± 0.042 (FB20) and from 0.75 ± 0.007 (KM0) to 2.79 ± 0.014 (KM30) in buns and muffins respectively. These results revealed that percentage increase for TDF was 272 per cent in KM30 and 268.92 per cent in F20. This higher fiber content of bran helped to reduce total carbohydrate (T.CHO) and available carbohydrate (A.CHO) in both the products compared to their controls. Reduction in T.CHO was 2.47 per cent and 1.77 per cent, whereas reduction in A.CHO accounted to 6.38 per cent and 6.18 per cent in buns and muffins respectively. These results agree with the previous studies where in protein, ash, fibre contents were increased when products (biscuits and breads) were value-added with bran (Jisha *et al.* 2010; Sairam *et al.* 2011 and El-Sharnouby *et al.* 2012).

4.9.2 Mineral content

Minerals are important for normal and healthy functioning of the human body. Since human body is unable to generate its own minerals, diet becomes a vital source. Thus, deficiencies may result if diet is lacking in minerals. Amongst all minerals major global concern of public health is iron and zinc deficiency. Calcium is another such mineral necessary for bone development and required for contraction and relaxation of muscles, and adequate consumption may reduce risk associated with hypertension. Sodium and potassium are important minerals maintaining electrolyte balance in body (Shankar, 2013). Considering all these factors iron, calcium, zinc, sodium and potassium contents were estimated in buns and muffins.

Table 4.24. Nutritional composition of designer products

Nutrients (g/100g)	Bun		Muffin	
	FB0	**FB20**	**KM0**	**KM30**
Moisture (g)	24.95 ± 0.197	24.84 ± 1.271	25.08 ± 1.588	25.40 ± 0.563
Protein (g)	5.65 ± 1.157	6.83 ± 0.804	6.96 ± 0.111	7.03 ± 0.303
Fat (g)	15.92 ± 1.138	15.71 ± 2.013	19.22 ± 0.123	19.29 ± 0.697
Ash (g)	1.63 ± 0.995	2.05 ± 0.048	1.23 ± 0.031	1.62 ± 0.005
TDF (%)	0.74 ± 0.009	2.73 ± 0.042	0.75 ± 0.007	2.79 ± 0.014
T. CHO (g) *	51.85 ± 0.994	50.57 ± 2.191	47.50 ± 1.795	46.66 ± 0.952
A. CHO (g) *	51.11 ± 0.986	47.85 ± 2.209	46.76 ± 1.799	43.87 ± 0.937
Energy (Kcal)*	373.31±10.067	371.01±8.722	390.84±6.189	388.36±1.938

Note: Values are expressed as Mean ± SD; * calculated value; FB0: control bun, FB20: 20 per cent foxtail bran bun; KM0: control muffin, KM30: 30 per cent kodo bran muffin

The results in Table 4.25 showed that iron, calcium, zinc and sodium content increased while potassium content lowered in bran enriched buns and muffins compared to their controls. The iron content of 100 g bran enriched muffins (KM30: 7.86 mg/100 g) was more than 1/3rd of the recommended dietary allowances (RDA) suggested for Indian adult man (19 mg/day) while buns (FB20: 13.83 mg/100 g) have more than 1/3rd of the RDA suggested for both Indian adult man (19 mg/day) and woman (29 mg/day). Zinc content showed less increase compared to iron in bran enriched buns (3.73 ± 0.208 to 4.53 ± 0.187 mg/100 g) and muffins (0.79 ± 0.019 to 0.85 ± 0.004 mg/100 g). Bran blended buns (23.90 mg/100 g) and muffins (40.68 mg/100 g) had slightly higher amount of calcium than control, and both products remained a good source of calcium. Sodium content increased in FB20 and KM30 compared to their respective control FB0 and KB30. The potassium content in muffins ranged from 65.01 ± 1.111 mg/100 g (KM30) to 76.08 ± 0.808 mg/100 g (KB0) and from 55.52 ± 0.256 mg/100 g (FB20) to 67.49 mg/100 g (FB0) in buns. These results indicated that bran blended buns and muffins had a good mineral profile, and both products were good source of iron.

The previous study also indicated that wheat bran enriched muffins (up to 24 %) had higher iron and calcium content than muffin with no bran (Romjaun and Prakash, 2013). Iron content of muffins in the present study was much higher while calcium content was similar compared to wheat bran enriched muffins developed in the previous study (Romjaun and Prakash, 2013). Zinc content was similar for muffins and much higher for buns developed in the present study than that of biscuits prepared with 30 per cent wheat bran in the previous study (Ertas, 2015).

Table 4.25. Mineral composition of designer products

Minerals (mg/100g)	Bun		Muffin	
	FB0	**FB20**	**KM0**	**KM30**
Iron (mg)	1.22 ± 0.090	13.83 ± 0.657	1.51 ± 0.053	7.86 ± 0.213
Zinc (mg)	3.73 ± 0.208	4.53 ± 0.187	0.79 ± 0.019	0.85 ± 0.004
Calcium (mg)	15.59 ± 0.209	23.90 ± 1.446	34.68 ± 0.371	40.68 ± 0.463
Potassium (mg)	67.49 ± 0.111	55.52 ± 0.256	76.08 ± 0.808	65.01 ± 1.111
Sodium (mg)	34.19 ± 0.216	45.60 ± 0.511	31.68 ± 0.090	35.98 ± 0.451

Note: FB0: control bun, FB20: 20 per cent foxtail bran bun; KM0: control muffin, KM30: 30 per cent kodo bran muffin; Values are expressed as Mean ± SD.

4.10 Antioxidant capacity and phytonutrient content of best accepted minor millet bran incorporated designer muffins and buns

Muffins (KM0, KM30) and bun (BM0, BM30) were evaluated for their phytonutrient content and antioxidant capacity. The results presented in Table 4.26 revealed that bran enriched muffins (KM30) and buns (FB20) contained higher quantity of phytonutrients and had better antioxidant capacity compared to control (KM0, FB0). These antioxidants contribute in prevention of various degenerative diseases caused by oxidative damage.

4.10.1 DPPH radical scavenging activity

The inhibition of DPPH (%) was higher in FB20 and KM30 compared to their respective control (FB0, KM0). In buns per cent inhibition of DPPH (%) increased from 7.06 ± 0.692 (FB0) to 19.17 ± 0.000 (FB20) which account to almost 2.7 times greater inhibition of DPPH in foxtail bran enriched buns (FB20) as compared to control buns (FB0) prepared using refined wheat flour. In muffins this increase was noted up to 2.16 times, where inhibition of DPPH (%) increased from 11.98 ± 0.923 (KM0) to 25.97 ± 2.537 (KM30). The results are in accordance with a previous study where pasta enriched with 20 per cent rice bran had highest reducing power and DPPH radical scavenging activity compared to pasta having no bran (Nithya *et al.* 2013). Hussein and Ibrahim

(2019) also showed that antioxidant activity increased with addition of rice, wheat and barley bran in breads.

4.10.2 Ferric Reducing Antioxidant Power (FRAP)

Antioxidant capacity in terms of FRAP assay also exhibited 3.9-fold increase in buns with foxtail bran, whereas the increase was 8.5-folds in muffins with kodo bran. FRAP (μM/g) values in buns increased from 229.00 ± 3.464 (FB0) to 894.33 ± 9.238 (FB20), where as in muffins from 100.67 ± 5.774 (KM0) to 861.67 ± 11.590 (KM30).

This increase in antioxidant capacity in terms of DPPH and FRAP can be associated with higher TP content in bran enriched buns and muffins compared to their respective controls. Similarly, previous studies also indicated a correlation between TP and antioxidant activities in bran enriched products (Nithya *et al.* 2013 and Hussein and Ibrahim, 2019). Bran and bran rich fraction are reported to possess a high concentration of phenolic compounds and other compounds like flavonoids and phytic acid that enhance antioxidant capacity in bran enriched products. In the present and in a previous study kodo and foxtail, bran have been reported to have excellent antioxidant profile as they contain flavonoids, phenol and phytic acid (Bijalwan *et al.* 2016).

4.10.4 Total phenols (TP)

TP (mg GAE/100 g) content increased up to 319.77 (FB20) and 402.40 (KM30) from 152.53 (FB0) and 224.80 (KM0). This increased TP content of bran enriched buns and muffins is due to addition of bran which is a concentrated source of phenols. Similar results for increased TP and antioxidant capacity in wheat, rice and barley bran enriched breads was reported by Hussein and Ibrahim (2019). Guo *et al.* (2018) also reported highest antioxidant capacity in terms of DPPH, FRAP and TEAC assay for wine prepared from foxtail millet bran due to presence of polyphenol content.

4.10.3 TBARS (Thiobarbituric Acid Reactive Substances) assay

In TBARS (μM/g) assay, lipid peroxidation values decreased from 1.19 ± 0.077 (FB0) to 0.85 ± 0.020 (FB20) in buns and 1.81 ± 0.106 (KM0) to 1.29 ± 0.049 (KM30) in muffins. This decrease in TBARS values is due to lower MDA production. MDA is produced during lipid peroxidation, if the lipid peroxidation is inhibited then less MDA is produced (Zeb and Ullah, 2016). These results indicate that bran enriched muffins and buns had higher antioxidant capacity as it helped in lowering lipid peroxidation effect thus lowering MDA production. Similarly, a previous study indicated that rice bran enriched breads had lower MDA formation compared to breads without rice bran indicating less peroxidation due to antioxidant effect of rice bran (Sairam *et al.* 2011).

4.10.5 Phytic acid (PA)

Presence of phytic acid is known to hinder absorption of minerals by binding with them, but it is also known for contributing to antioxidant capacities (Abdulwaliyu *et al.* 2019). In the present study, phytic acid increased from 599.67 ± 6.110 (F0) to 792.67 ± 6.526 (F20) in buns and 488.76 ± 8.68 (K0) to 650.00 ± 8.660 (K30) in muffins. This increase in phytic acid might have contributed to lowering peroxidation of lipids (Graf, 1990 and Abdulwaliyu *et al.* 2019), thus increasing bran enriched products' antioxidant capacity.

Thus, it was observed from proximate, mineral, antioxidant and phytonutrient content that addition of bran to refined wheat flour muffins and buns, resulted in a better nutrient profile compared to their counterparts. Thus, bran can be utilised as a healthy functional ingredient in the food industry, especially to enhance the nutritional value of the empty calorie products like baked snacks including buns, bread, muffins and biscuits etc. Brans being rich in phytonutrients and antioxidants, they can be explored in nutraceutical preparation.

Table 4.26. Antioxidant and phytonutrient composition of designer products

Parameter	**Bun**		**Muffin**	
	FB0	**FB20**	**KM0**	**KM30**
DPPH (%)	7.06 ± 0.692	19.17 ± 0.000	11.98 ± 0.923	25.97 ± 2.537
FRAP (µM/g)	229.00 ± 3.464	894.33 ± 9.238	100.67 ± 5.774	861.67± 11.590
TBARS (µM/g)	1.19 ± 0.077	0.85 ± 0.020	1.81 ± 0.106	1.29 ± 0.049
TP (mg GAE/100g)	152.53 ± 4.272	319.77 ± 8.025	224.8 ± 13.683	402.40 ±12.471
PA (mg /100g)	599.67 ± 6.110	792.67 ± 6.526	488.76 ± 8.68	650.00 ± 8.660

Note: FB0: control bun, FB20: 20 per cent foxtail bran bun; KM0: control muffin, KM30: 30 per cent kodo bran muffin; Values are expressed as Mean ± SD.

4.11 Shelf life of best accepted minor millet bran incorporated designer muffins and buns

Designer muffins and buns along with their controls were stored in room temperature for 7 days and assessed at interval of 0, 3rd and 7th day. The parameters evaluated were moisture, water activity, microbial load and sensory quality. In buns visible spoilage was observed on 4th day of storage, leaving buns unfit for consumption.

4.11.1 Moisture content

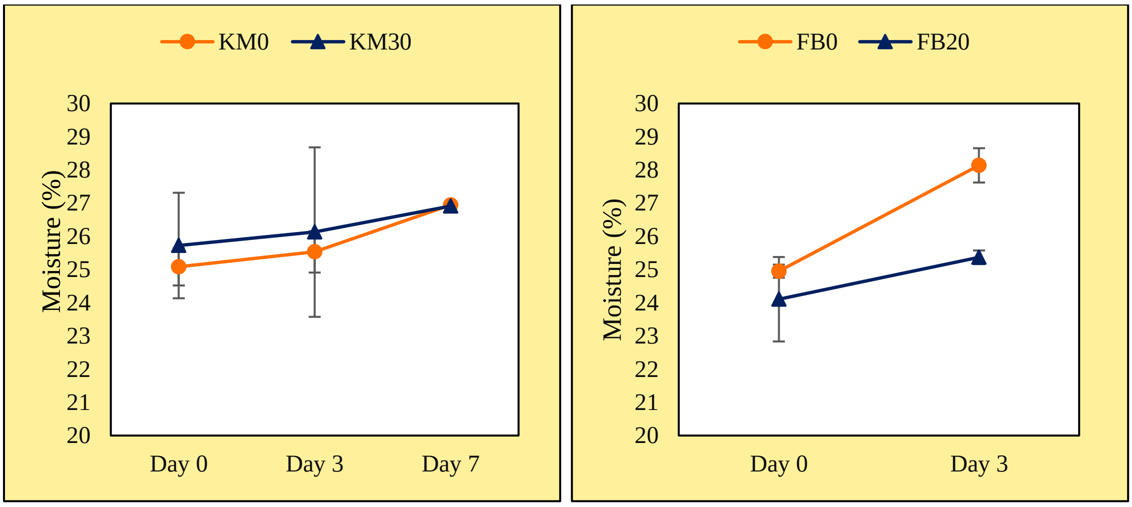
Two factor ANOVA was conducted to study the interaction of treatments and storage period on moisture content, but there was no significant difference in the moisture content of muffins with and without bran addition (KM0, KM30). The moisture (%) content increased from 25.08, 25.72 (day 0) to 26.94, 26.91 (day 7) for KM0 and KM30 respectively (Fig 4.11 a). In buns, treatment means showed that control (FB0) had significantly higher moisture content compared to FB20 which increased significantly ($p<0.05$) in both treatment (FB0, FB20) after 3 days of storage. Moisture (%) content increased from 24.95 (day 0) to 28.14 (day 3) in FB0 whereas from 24.11 (day 0) to 25.37 (day 3) in FB20 (Fig 4.11 b).

Overall, the results suggested that moisture content of buns and muffins increased significantly by end of the storage period. It was observed from a previous study that moisture content usually decreased in the crumb, but increases in crust (Ho *et al*. 2014). The samples used in present study were mixture of crust and crumb; thus, the noted increase in moisture content during storage period could be due redistribution of moisture from center core of crumb. Similar results of increased moisture content during 3 days of storage in bread crust samples were reported by Ho *et al*. (2014). However detailed experiments are required to understand the moistures' role in present baked products' shelf life.

4.11.2 Water activity

Water activity is an important factor in determining the shelf life of products. Higher the water activity higher are the chances of spoilage. Typically, sponge cakes have water activity ranging from 0.91 – 0.87; snack cakes have 0.65 – 0.75 and breads have 0.93 – 0.98 (Fontana, 2000 and Frazier *et al.*, 2014).

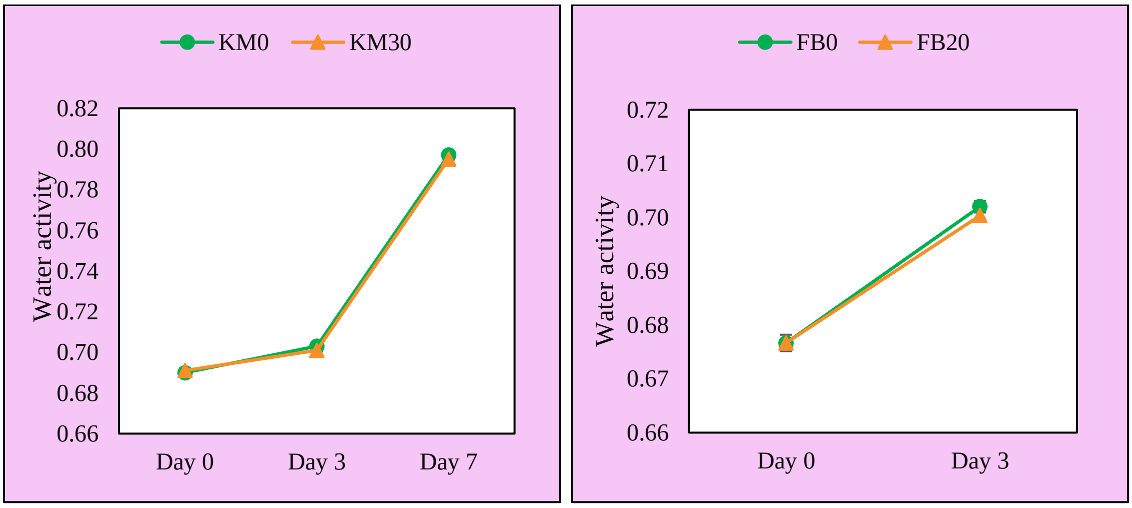
Water activity during storage significantly increased from 0.690 (0 day) to 0.797 (7th day) in K0 and 0.691 (0 day) to 0.795 (7th day) in K30 but no significant difference was noted between the treatments (Fig 4.12 a). Similarly, in buns the treatments F0 and F20 showed no significant difference, but significant increase was observed during storage period. The increase was noted from 0.677 (F0, F20) at day 0 to 0.702, 0.700 (F0, F20) at 3rd day storage (Fig 4.12 b). Increased water activity is associated to increased presence of unbound water in food, favoring microbial growth (Nielsen *et al*. 2012).



(a) (b)

Fig 4.11 Moisture content of muffins (a) and buns (b) during storage

Note: KM0: control muffin, KM30: 30 per cent kodo bran muffin; FB0: control bun, FB20: 20 per cent foxtail bran bun



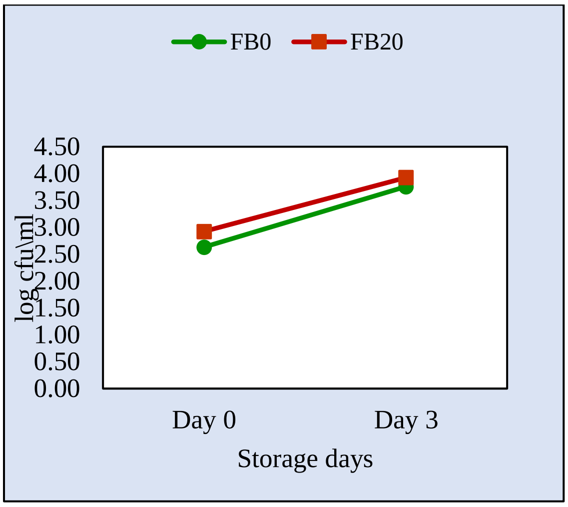
(a) (b)

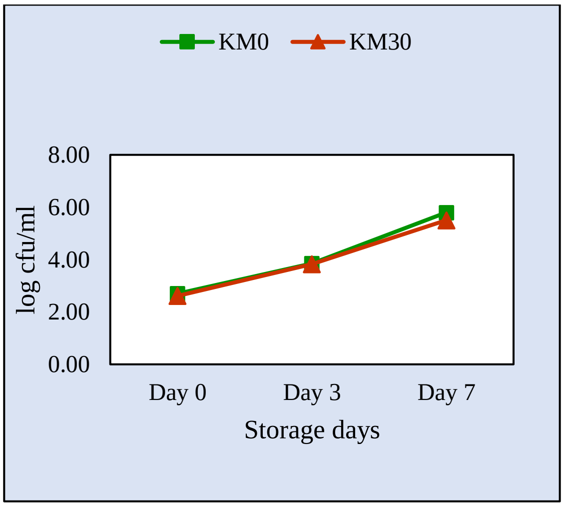
Fig 4.12 Water activity of muffins (a) and buns (b) during storage

Note: KM0: control muffin, KM30: 30 per cent kodo bran muffin; FB0: control bun, FB20: 20 per cent foxtail bran bun

4.11.3 Microbial load

In the present study total bacterial count (TBC) and total mould count (TMC) increased during storage of 3 (buns) and 7 days (muffins). TBC (log cfu/ml) in buns increased from 2.63 (FB0), 2.92 (FB20) on day 0 to 3.76 (FB0), 3.93 (FB20) on 3rd day of storage (Fig 4.13 a). Similarly, in muffins, TBC increased from 2.70 to 5.80 in KM0 and 2.61 to 5.51 in KM30 during 7 days of storage (Fig 4.13 b). TMC (log cfu/ml) also increased up to 2.15 (FB0), 2.30 (FB20) in buns during 3 days of storage and up to 3.57 (KM0), 3.73 (KM30) during 7 days of storage (Fig 4.14 a, b). Increase in TBC and TMC during storage was well within the documented limits given by FSANZ (2016). Similar increase in microbial plate count was observed on extruded snacks incorporated with rice, wheat and oat bran during storage (Dar *et al.* 2016). Increase in microbial population of bread samples after 3rd day was also reported in a previous study (Ayub *et al.* 2003). This increase in TBC and TMC could be attributed to increase in moisture and water activity during storage. Presence of moisture content and water activity favors the microbial growth on products. Visible spoilage was noted on buns on 4th day of storage indicating that buns had shelf life for 3 days only.

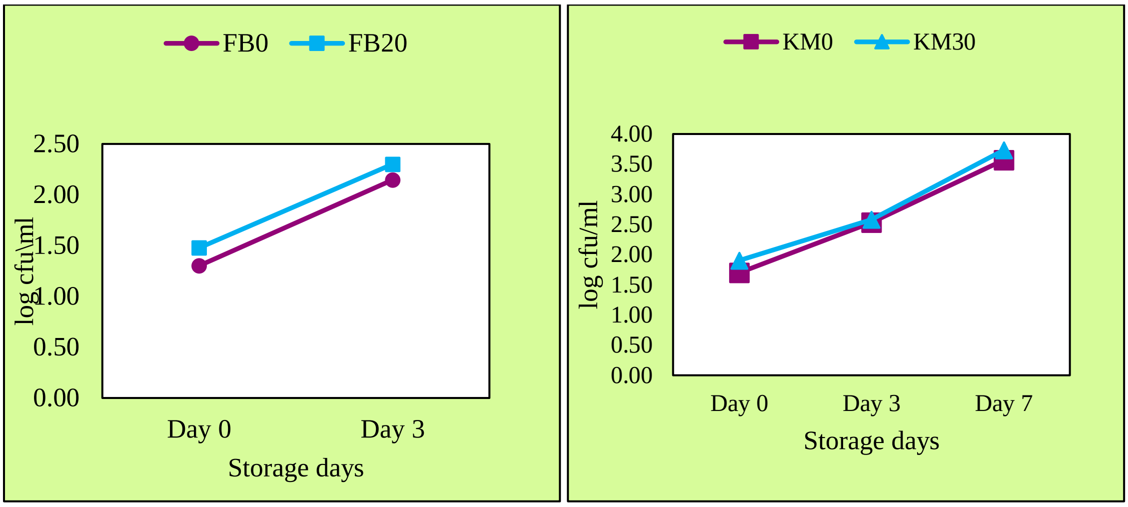




(a) (b)

Fig 4.13 Total bacterial count of buns (a) and muffins (b) during storage

Note: KM0: control muffin, KM30: 30 per cent kodo bran muffin; FB0: control bun, FB20: 20 per cent foxtail bran bun



(a) (b)

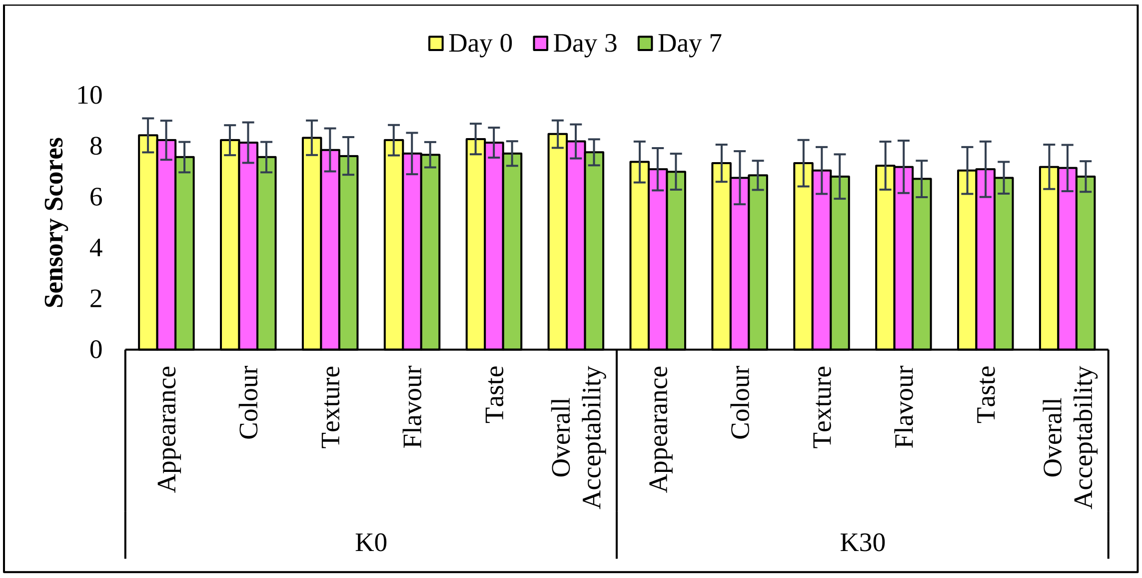
Fig 4.14 Total mould count of buns (a) and muffins (b) during storage

Note: KM0: control muffin, KM30: 30 per cent kodo bran muffin; FB0: control bun, FB20: 20 per cent foxtail bran bun

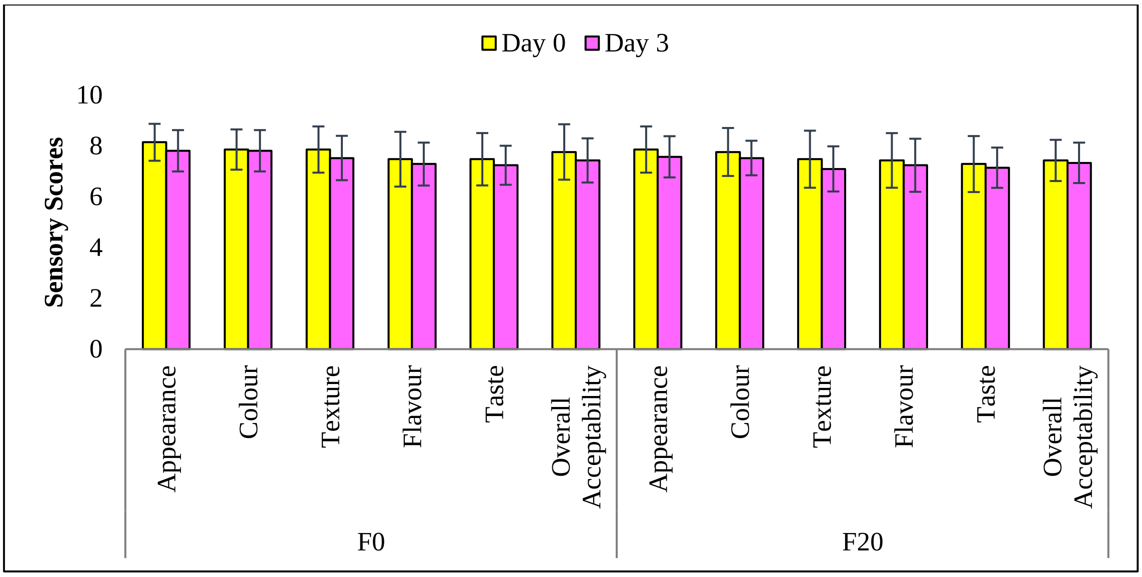
4.11.4 Sensory evaluation

A decreasing trend was observed in the sensory scores for both buns (FB0, FB20) and muffins (KM0, KM30) during the storage period (Fig 4.15). However, there was sensory scores for control (KM0) were higher compared to KM30 by end of storage period. Overall acceptability scores for KM0 decreased from 8.48 to 7.76 whereas in KM30 scores decreased from 7.19 to 6.81 during storage period (Fig 4.15 a). Scores for all parameters like appearance, color, texture, flavor, overall acceptability significantly decreased on 7th day of storage, but no significant difference was observed in scores for taste though a reduction was noted during storage.

A decreasing trend was observed in sensory scores for FB0 and FB20 during 3 days of storage (Fig 4.15 b). Overall acceptability decreased during 3 days storage from 7.76 to 7.43 and 7.43 to 7.33 in FB0 and FB30 respectively. No significant difference was observed in sensory quality of both control and foxtail bran enriched bun during storage ($p<0.05$).



(a)



(b)

Fig 4.15 Sensory scores of muffins (a) and buns (b) during storage

Note: KM0: control muffin, KM30: 30 per cent kodo bran muffin; FB0: control bun, FB20: 20 per cent foxtail bran bun

The decrease in sensory scores may be due to changes in texture, taste and flavor of buns and muffins. Twenty-one semi-trained panel members evaluated the products, and they reported hard texture, decreased sponginess, slight off flavor for during storage. In muffins, the scores for appearance, color, texture, flavor, and overall acceptability decreased significantly ($p<0.05$) by the end of storage period. In buns decrease in sensory scores was observed but it was not significant during storage period of 3 days. Decrease in sensory scores on storage for bran enriched products is due to slight off-flavor associated with increased lipid peroxidation in products (Sharif *et al.* 2009; Nagi *et al.* 2012 and Kamaljit *et al.* 2011).

The results of moisture, water activity microbial count and sensory palatability of buns and muffins during storage indicated that buns had a shelf life of three days while that of muffins had seven days when stored at room temperature. Similar results for bread and muffins samples stored at room temperature were recorded in previous studies (Ayub *et al.* 2003; Kamaljit *et al.* 2011 and Romjaun and Prakash, 2013).

4.12 Therapeutic potential of best accepted minor millet bran incorporated designer muffins and buns

To understand the therapeutic benefits, bran enriched buns (FB20) and muffins (KM30) were analyzed for glycemic index (GI) and glycemic load (GL). Single arm supplementation study was also conducted for 45 days with two groups (adolescent girls) for bun (n = 15) and muffin (n = 14). In the supplementation studies blood samples were collected pre- and post-feeding and analyzed for glucose, lipid and stress profile. Under stress profile lipid peroxidation, catalase and glutathione reductase were analyzed.

4.12.1 Glycemic index (GI) and Glycemic load (GL)

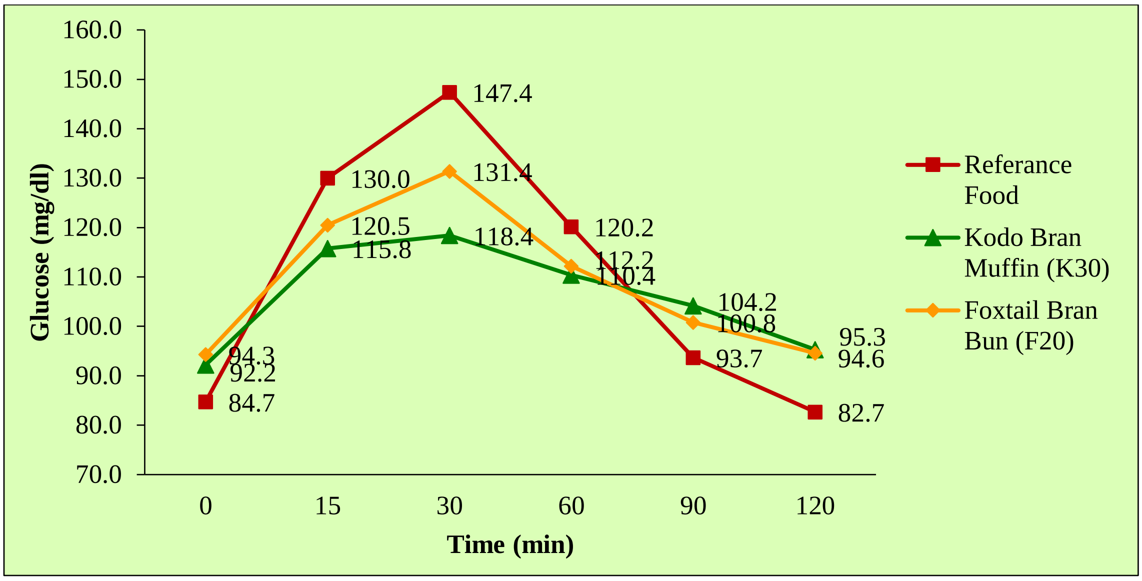
Based on blood glucose response after the consumption carbohydrate-based foods are classified using the concept of the glycemic index (GI) under low (<55), medium/intermediate (55 – 70) and high (>70) categories (Venn and Green, 2007 and Prasad *et al.* 2015). GI was calculated from postprandial blood glucose responses and incremental area under the curve (iAUC). It is established by researchers that various food products having same amount of carbohydrate can have different blood glucose responses and iAUC after consumption, depending on the composition of the food. Varietal differences in grains, quality and quantity of carbohydrate, size and structure of starch, dietary fiber, protein, lipid and phytonutrients like phytates, phenolic compounds as well as cooking method and time affects the GI (Dodd *et al*. 2011; Khawaja *et al.* 2012; Sieri *et al.* 2013; Soong *et al.* 2015; Eleazu, 2016 and Ganesan and Xu, 2017).

Changes in snacking habits and preference of starch rich and baked products like muffins and buns with higher glycemic index are alarming and increases risk of obesity, diabetes and other life style-related disorders. It has also been reported that consistently following diet patterns including lower GI foods is beneficial in lowering the risk of such life style disorders (Sanz-Penella *et al.* 2014). Glycemic load (GL) is also very vital in understanding the glycemic response of food along with GI. GL considers the amount of carbohydrate in the portion size of food consumed which is not considered in GI. GL categorizes foods into low (<10), medium (10 – 20) and high (>20) GL (Venn and Green, 2007). Considering all these factors designer products were evaluated for both their GI and GL.

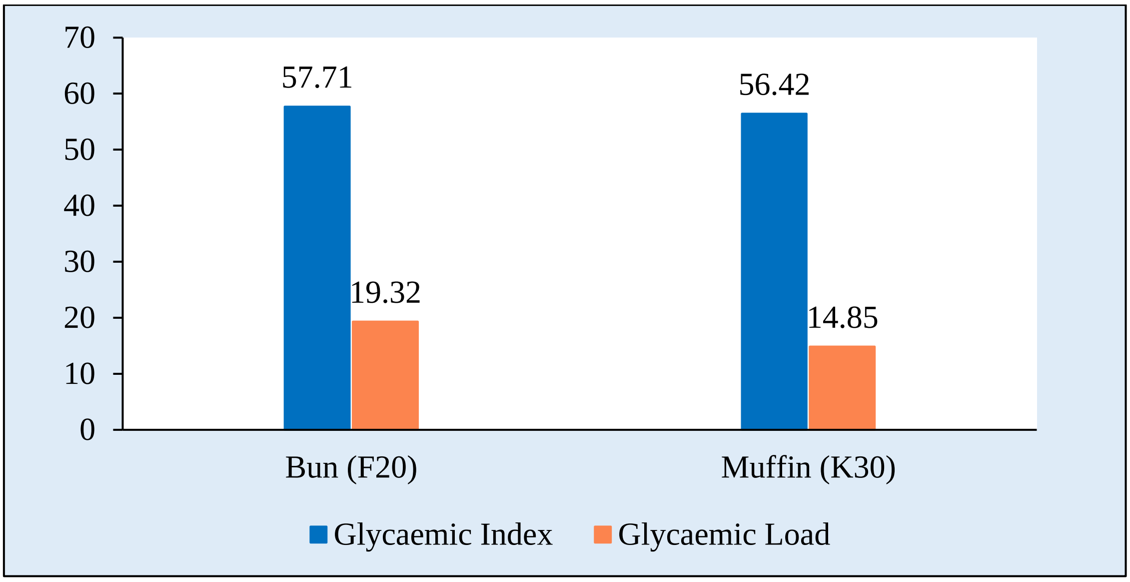
The results for blood glucose response for both bun (FB20) and muffins (KM30) compared to reference food (glucose) are depicted in Fig 4.16 a. These results suggested that in all foods peak rise in blood glucose levels was seen after 30 mins of consumption, but surge was lowest in KM30 (118.4 mg/dl), followed by FB20 (131.4 mg/dl) and reference food (147.4 mg/dl) showed highest rise. Similarly, Tosh *et al.* (2008) reported a reduction in peak blood glucose rise in muffins enriched with oat bran. GI and GL value for FB20 and KM30 are showed in Fig 4.16 b. The GI of buns in the present study is much lower than breads developed with wheat bran incorporation at 20 per cent (Sanz-Penella *et al.* 2014). The GI for muffins in present study was closer with that of muffins prepared using oat and barley flours in a previous study (Soong *et al.* 2015).

Generally, muffins and buns have high glycemic index as they are mainly made up of refined wheat flour. Still, the addition of bran might have contributed for the moderate GI of buns and muffins due to presence of dietary fiber and phytonutrients like total polyphenols and phytic acid (Eleazu, 2016 and Ganesan and Xu, 2017). Presence of dietary fiber and resistant starch in foods have been associated with hypoglycemic effect as they are not digested in small intestine, contributing very low to blood glucose rise after consumption (Krawęcka *et al.* 2019). Similarly, previous studies have also indicated that bran or dietary fiber reduced amount of available carbohydrate thus, reducing the GI showing hypoglycemic effect (Venn and Green, 2007; Tosh *et al.* 2008 and Soong *et al.* 2015). Another cross-over study also indicated that addition of oat β-glucan from oat bran to instant oat meal reduced post prandial blood glucose levels after supplementation in dose-dependent manner (Wolever *et al.* 2018).

In the present study, portion sizes of 60 g (2 no.) and 70 g (1 no.) for muffins (KM30) and buns (FB20) respectively were considered as one serving to calculate GL. The results revealed that GL for one serving of 60 g muffin (KM30) was 14.85 and for one serving of 70 g bun (FB20) was 19.32 both products falling under category of medium GL foods (Fig 4.16 b).



(a)



(b)

Fig. 4.16 Mean blood glucose response (a) and glycemic index and glycemic load (b) of foxtail bran bun (FB20) and kodo bran muffin (KM30)

4.12.2 Effect of supplementation of minor millet bran incorporated designer muffins and buns in adolescents

Muffins and buns were supplemented daily to 2 independent groups for 45 days. Initially, 16 members were assigned randomly in muffins groups and 15 in buns group. Depending on the group each member consumed either buns (FB20) or muffins (KM30) daily as breakfast or snack for 45 days. Muffins and buns were supplemented in addition to their daily diet. During study 2 participants from muffins group left the study due to their personal reasons. This resulted in 14 as the final number of subjects in muffin group. This study's motive was to evaluate therapeutic effect of bran enriched buns and muffins without altering daily diet of subjects. Various parameters like blood glucose level, lipid profile, protein level and stress profile (lipid peroxidation, glutathione reductase and RBC catalase) were analyzed before and after the supplementation.

4.12.2.1 Characteristics of the subjects

The profile of subjects selected for both the groups is presented in Table 4.27. Both the groups had female members studying BSc. Agriculture (1st year) residing in hostel campus. Average age (years) of participants in muffin and bun group was 18.93 ± 0.73 and 18.80 ± 1.01 with average height of 1.56 ± 0.05 m, 1.55 ± 0.06 m and weight 49.46 ± 6.95 kg, 50.40 ± 5.03 kg respectively. The average BMI (kg/ m^2) fell under normal category for both muffin (20.22 ± 1.96) and bun (20.87 ± 2.05) groups (WHO, 2004).

The muffins group's general food habits showed that all the participants (100 %) were non-vegetarian whereas in bun group 86.67 per cent were non-vegetarian and 13.33 per cent were vegetarian. It also revealed that most subjects followed 3 – 4 meal pattern (92.86%, 86.67%) and only 7.14, 13.33 per cent followed 1 – 2 meal patterns in both muffin and bun group respectively. The subjects who recorded 1 – 2 meal pattern frequently missed breakfast and only consumed lunch and dinner. The motive behind selecting hostel residents was uniformity in the meals served in hostel, as in this study no additional control was exercised on daily meal pattern or foods consumed by the subjects.

In addition to hostel meals data regarding frequency of consuming outside food was recorded and it was revealed that 28.57 per cent (muffin group), 33.33 per cent (bun group) subjects consumed at least once in 15 days. Whereas 35.71 per cent, 13.33 per cent ate outside once in a week and 35.71 per cent, 40 per cent ate outside twice a week in muffins and buns group respectively. In bun group 2 members (13.33 %) recorded consuming outside food daily. While purchasing foods/snacks outside only 2 (14.29 %) members from muffin group and only 1 (6.67 %) member from bun group reported that they look for nutrition information or health benefits, rest 12 (85.71 %) from muffin and 14 (93.33 %) members from bun group did not look for any such information.

When questioned about millet consumption only, 1 participant from bun group reported consumption of jowar, bajra (1 – 2 times per week) and ragi (1 – 3 times per month), whereas from muffin group 1 participant reported only consumption of jowar (1 – 2 times per week). Majority of subjects *i.e.,* 64.29 per cent (muffin group), 53.33 per cent (bun group) reported that they do not follow any regular exercise pattern and only 35.71 per cent (muffin group) and 46.67 per cent (bun group) did regular exercise.

Table 4.27. General characteristics of subjects in muffins and bun groups

Variables	Muffin group (n=14)	Bun group (n=15)
General characteristics		
Age (years)	18.93 ± 0.73	18.80 ± 1.01
Education	1st year BSc (Agriculture)	1st year BSc (Agriculture)
Height (m)	1.56 ± 0.05	1.55 ± 0.06
Weight (kg)	49.46 ± 6.95	50.40 ± 5.03
BMI (kg/m^2)	20.22 ± 1.96	20.87 ± 2.05
General food habits		
Vegetarian (no.)	0	2 (13.33%)
Non-vegetarian (no.)	14 (100%)	13 (86.67)
Number of meals consumed per day		
1 – 2 meals	1 (7.14%)	2 (13.33%)
3 – 4 meals	13 (92.86%)	13 (86.67)
How often do you eat outside		
Once in 15 days	4 (28.57%)	5 (33.33%)
Once in week	5 (35.71%)	2 (13.33%)
Twice in week	5 (35.71%)	6 (40%)
Daily	0	2 (13.33%)
Do you look for nutrition / health information of foods/snacks while purchasing		
Yes	2 (14.29%)	1 (6.67%)
No	12 (85.71%)	14 (93.33%)
Do you exercise regularly		
Yes	5 (35.71%)	7 (46.67%)
No	9 (64.29%)	8 (53.33%)

Note: values are expressed as mean ± SD for general characteristics and as percentage (%) for general food habits

Frequency of most commonly consumed bakery items by subjects of both groups were recorded and presented in Table 4.28. The results showed that from bun group only 7.14 per cent (1 member) consumed buns 1 – 3 times/month, while 92.86 per cent (13 members) rarely consumed buns. In muffin group also highest number of participants 11 (73.33 %) never or rarely consumed buns while 3 (20.00 %) participants consumed 1 – 3 times/month and only 1 (6.67 %) participant consumed 1 – 2 times/week. Bread consumption was reported by 42.86 per cent and 53.33 per cent subjects in muffin and bun group respectively. Compared to muffin/cupcake, cake or pastries where commonly consumed (1 – 3 times/month) by subjects in both the groups. Biscuit was the frequently consumed bakery product by all the participants. Pizza and burger were other commonly consumed bakery snacks (1 – 3 times/month) by 64.29 per cent and 66.79 per cent of participants from muffin and bun group respectively. These results communicate that consumption of common bakery items especially biscuits, cake/pastries and burger/pizza has increased in adolescents with a frequency of 1 – 3 times/month.

4.12.2.2 Blood glucose levels

Consumption of high fiber diet, cereal fiber rich diets, whole grain and bran mixed diets have been associated with reducing the risk of obesity, cardiovascular diseases, type 2 diabetes (Koh-Banerjee *et al.* 2004; FDA, 2008; Lattimer and Haub, 2010; Cho *et al.* 2013 and Johansson-Persson *et al.* 2014). Consumption of whole grains, or dietary fiber rich diet reduces the glycemic index of diet and has been associated with its hypoglycemic effect by delaying transit time and delaying absorption of glucose (Krawęcka *et al.* 2019).

In the present study Table 4.29 denotes blood glucose level in both muffin and bun group before and after supplementation of 45 days. Blood glucose levels reduced significantly ($p<0.05$) in bun group whereas the reduction was insignificant in muffin group after 45 days. Bran consists of indigestible carbohydrate like dietary fiber that led to increased dietary fiber content of muffins and buns. The presence of these dietary fibers in muffins and buns can be attributed to beneficial effect on reducing the blood glucose levels after continuous consumption for 45 days. These dietary fibers cannot be digested in stomach and are fermented in colonic region by gut microbiota resulting in the formation of short chain fatty acids. According to literature these short chain fatty acids may have beneficial effects on glucose metabolism (Nilsson *et al.* 2006).

Table 4.28. Frequency of consumption of bakery products among subjects recruited in muffins and bun groups

Frequency of consumption of bakery products among subjects recruited in Muffin group (n=14)							
Number (%)							
Item	**Never or rarely**	**1–3 per month**	**1–2 per week**	**3–4 per week**	**5–6 per week**	**Daily**	**2–3 per day**
Buns	13(92.86)	1(7.14)	-	-	-	-	-
Breads	8(57.14)	6(42.86)	-	-	-	-	-
Muffin/ cupcake	9(64.29)	5(35.71)	-	-	-	-	-
Cake/ Pastries	2(14.29)	11(78.57)	1(7.14)	-	-	-	-
Biscuits	-	10(71.43)	1(7.14)	1(7.14)	-	1(7.14)	1(7.14)
Toast	13(92.86)	1(7.14)	-	-	-	-	-
Burgers/ Pizza	5(35.71)	9(64.29)	-	-	-	-	-
Frequency of consumption of bakery products among subjects recruited in Bun group (n=15) n (%)							
Number (%)							
Item	**Never or rarely**	**1–3 per month**	**1–2 per week**	**3–4 per week**	**5–6 per week**	**Daily**	**2–3 per day**
Buns	11(73.33)	3(20.00)	1(6.67)	-	-	-	-
Breads	7(46.67)	8(53.33)	-	-	-	-	-
Muffin/ cupcake	12(80.00)	3(20.00)	-	-	-	-	-
Cakes/ Pastries	1(6.67)	14(93.33)	-	-	-	-	-
Biscuits	1(6.67)	3(20.00)	7(46.67)	3(20.00)	1(6.67)	-	-
Toast	10(66.67)	5(33.33)	-	-	-	-	-
Burgers/ Pizza	5(33.33)	10(66.67)	-	-	-	-	-

Though there was a reduction in the blood glucose levels it was not significant in muffins but significant in bun group, this can be attributed to factors such as individual variations, the medium used for supplementation, its overall composition (fat and sugar content) can affect the glucose responses. Thus, muffins developed with higher sugar and fat showed insignificant results compared to that of buns with lower fat and sugar content. Individual variations, medium used for supplementation, composition of food other than fibre content, cooking methods and procedures can have major impact on foods' glucose responses (Willis *et al.* 2011).

Overall results in the present study showed that blood glucose levels did not exceed and maintained well within the normal levels. Instead, a reduction was observed despite no control being exercised over the other foods consumed by subjects in both the groups. There was no adverse effect of bun or muffin consumption for 45 days. This reduction in blood glucose levels can be the attributed to combined effect of fiber and phytic acid component in bran that regulated glucose-regulating enzyme activity. Similar findings of significant reduction in blood glucose levels have been reported by Kim *et al.* (2010) in groups of mice fed with high fat diet supplemented with rice bran or phytic acid when compared to mice fed only with high fat diet or the control group.

Thus, it can be suggested that consumers of buns and muffins as snacks can replace regular refined wheat flour muffins and buns with bran enriched ones having better dietary fiber profile to avoid consequences due to high fat and high sugar consumptions. Studies conducted previously have also reported a significant decrease in blood glucose response of wheat or jowar bran enriched *papad* in non-insulin-dependent diabetic subjects after 3 months of consumption (Kamble and Shinde, 2004).

Table 4.29. Effect of supplementation (45 days) on blood glucose levels

Blood glucose (mg/dl)						
Group	**Pre**	**Post**	**df**	**t stat**	**t critical**	**p-value**
Muffin	78.79 ± 6.142	78.07 ± 4.665	13	0.552	2.160	0.590^{NS}
Bun	80.60 ± 6.812	78.07 ± 4.350	14	2.371	2.145	0.033^{*}

Note: Values are expressed as means ± SD; Muffins: n = 14, Buns: n =15; df: degree of freedom; *significance at 5%; NS: non-significant.

4.12.2.3 Blood lipid profile

Studies have demonstrated that high fiber diet (48 g dietary fiber) reduced C-reactive protein thus, can reduce the risk of cardiovascular diseases (Johansson-Persson *et al.* 2014). Diet rich in dietary fiber (25 – 35 g/day including 6 g soluble fiber) obtained from fruits, vegetables and whole grain with decreased fat consumption (saturated fats and cholesterol) can reduce the risk of coronary heart diseases (FDA, 2008 and Lattimer and Haub, 2010). Thus, in the present supplementation study, blood samples were analysed (pre and post) to evaluate the bran enriched muffins and buns on the lipid profile of subjects. Paired t – test was used to identify the significance of the experiment and the results for lipid profile are presented in Table 4.30. It was observed from the results that there were no significant ($p>0.05$) changes in the total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), TC to HDL ratio. The very low-density lipoprotein VLDL and triglycerides (TG) increased significantly ($p<0.05$) during the study period in both groups, however it remained within the normal levels.

Results show insignificant decrease in HDL (mg/dl) level from 44.93 ± 10.644 (pre-test) to 43.57 ± 8.600 (post-test) for muffins group, whereas insignificant increase from 47.67 ± 9.325 (pre-test) to 49.00 ± 6.708 (post-test) in bun group indicating that consumption of bran enriched muffins and buns may not have significantly increased the HDL levels but helped in maintaining them in optimal range (>40 - 60 mg/dl). Desirable levels of TC are <200 mg/dl which were maintained in both the groups. Though statistically insignificant, TC (mg/dl) decreased in muffins group from 149.07 ± 22.362 (pre-test) to 145.21 ± 24.141 (post-test), but increased in bun group from 152.13 ± 30.253 (pre-test) to 155.33 ± 22.372 (post-test). LDL (mg/dl) also showed a decrease after 45 days supplementation, although it was not statistically significant ($p>0.05$). This shows that longer duration studies are required to analyse effect of bran enriched muffins and buns on lipid profile and obtain concrete results.

An unexpected increase in TG and VLDL after 45 days supplementation in both the groups was bewildering. This inconsistency observed in the lipid profile despite all values being in normal limits, emphasizes the fact that this variation can be due to individual variations and no control conditions on the additional diet like deep fired oily foods, manchurian, noodles, *pakoda*, fried chips etc. that have high fat content consumed by participants of both the groups during the course of study. Similarly, Althwab (2013) stated that no significant effect on overweight college students' blood lipid profile was observed after wheat bran muffin supplementation due to lack of control over differences in the participants' diets, the lesser duration and sample size. On the contrary, Tong *et al.* (2014) documented reduction in plasma TC and LDL in selected hypercholesterolemic hamsters after feeding dietary wheat bran arabinoxylans (5 g/kg) for 30 days, as the study was conducted under laboratory conditions with controlled animal diets.

Along with the above stated reasons, increase in TG and VLDL might also be due to the fat composition of muffins (19.29 g/ 100 g) and buns (15.71g / 100 g) developed in the present study. As one serving portion with 60 g muffins and 70 g buns had 11.57 g and 11.00 g of fat, contributing nearly half of recommended daily visible fat consumption for Indian sedentary man and woman (ICMR, 2020). In another study it was observed that consumption of oat bran along with regular jogging exercise had beneficial effect on lipid profile with significant decrease in TC, TG, LDL and significant increase in HDL in sedentary male, whereas insignificant increase was observed in TG and HDL in group only fed with oat bran and no exercise (Sahrir *et al.* 2017). Thus, even in the present study the increase in VLDL, TG and no significant increase in HDL may be because more than half of the subjects (64.29 % in muffins group and 53.33 % in buns group) did not do any regular exercise. The study conducted by Ooi and Ridzuan (2016) also supported that significant reduction in LDL, TG and TC was observed in group where oat bran supplementation was combined with exercise, instead of oat bran supplementation alone.

Another important parameter is non-HDL cholesterol used as screening tool to assess the cardiovascular diseases' risk (Virani, 2011). In adolescents the values below 120 mg/dl are considered as normal and acceptable (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, and National Heart, Lung, and Blood Institute, 2011). It was observed in the present study that in bun and muffin group these values were well within the normal levels. The non-HDL cholesterol (mg/dl) decreased from 104.14 ± 20.508 to 101.64 ± 25.267 in muffin group and increased from 104.47 ± 26.811 to 106.33 ± 20.325 in bun group (Table 4.30) though not significant ($p>0.05$).

TC to HDL ratio, TG to HDL ratio, LDL to HDL ratio and LDL to VLDL ratio are other important factors that help in assessing and predicting the risk of cardiovascular disorders (Millán *et al.* 2009 and Shim *et al.* 2016). The values for TC–HDL ratio in the present study were within the advisable limits (3.0 – 5.0). In both muffin (from 3.36 ± 0.803 to 3.33 ± 0.814) and bun (from 3.27 ± 0.706 to 3.18 ± 0.442) group, non-significant decrease was noted in TC–HDL ratio after the supplementation period of 45 days (Table 4.30). With regard to TG-HDL ratio significant increase was noted in bun group from 1.34 ± 0.401 to 1.51 ± 0.359 whereas the increase was insignificant in muffin group (1.79 ± 1.040 to 2.17 ± 1.241). Though the increase was noted in TG-HDL ratio it was below the risk and well within the recommended levels. Increased levels of TG–HDL ratio (>2.27) is associated with increased cardiovascular disorder (Shim *et al.* 2016). TG-HDL ratio above 2.27 in obese white children and adolescent showed increased risk of insulin resistance (Giannini *et al.* 2011). The LDL to HDL ratio above 3.5 and 3.0 is considered as risk factor for men and women respectively (Millán *et al.* 2009).

In the present study the LDL–HDL ratio decreased from 2.09 ± 0.633 to 1.97 ± 0.631 (muffin group) and 1.99 ± 0.672 to 1.90 ± 0.418 (bun group) after 45 days of supplementation (Table 4.30). It was noted that though the decrease was not significant ($p>0.05$) supplementation of both bran enriched muffins and buns helped in maintaining the LDL–HDL ratio within recommended ratio. LDL–VLDL ratio reduced significantly in both muffin (6.74 ± 2.394 to 5.38 ± 2.303) and bun (7.69 ± 2.255 to 6.40 ± 1.294) group after supplementation of 45 days. Overall, the results indicated that bran enriched buns and muffins might help in maintaining normal and healthy blood lipid profile in adolescents but longer duration studies are needed to establish the beneficial effects.

4.12.2.4 Blood protein and albumin content

Protein and albumin levels of designer buns and muffins supplemented pre- and post-blood samples are presented in Table 4.31. It was observed from the studies that supplementation of muffin (KM30) and buns (FB20) in both the groups decreased total protein and albumin levels significantly ($p<0.05$). This might be due to the factor that there was no control on participants' external eating habits or meals and individual variations. Further both kodo and foxtail bran might have hindered the protein and albumin absorption. Feeding hypercholesterolemic rats with wheat bran fiber for 30 days decreased total protein and albumin and authors opined that it inhibited protein synthesis and affected amino acid metabolism (Liu *et al.* 2014). Similar studies in rat have suggested that net protein ratio decreased significantly when rats were fed with wheat bran and guar gum fiber and in the study it was also suggested that protein utilization was negatively affected when fiber consumption alone was increased without protein in rats, as it led to increased excretion of endogenous faecal nitrogen (Shah *et al.* 1982).

Table 4.30. Effect of supplementation (45 days) on blood lipid levels

Total cholesterol – TC (mg/dl)						
Group	**Pre**	**Post**	**df**	**t stat**	**t critical**	**p-value**
Muffin	149.07±22.362	145.21±24.141	13	0.800	2.160	0.438 NS
Bun	152.13±30.253	155.33±22.372	14	-0.876	2.145	0.396 NS
High Density Lipoprotein – HDL (mg/dl)						
Muffin	44.93 ± 10.644	43.57 ± 8.600	13	0.737	2.160	0.474 NS
Bun	47.67 ± 9.325	49.00 ± 6.708	14	-0.883	2.145	0.392 NS
Low Density Lipoprotein – LDL (mg/dl)						
Muffin	89.21 ± 19.439	82.29 ± 20.480	13	2.067	2.160	0.059 NS
Bun	92.07 ± 25.516	91.73 ± 18.664	14	0.111	2.145	0.913 NS
Very Low-Density Lipoprotein – VLDL (mg/dl)						
Muffin	14.64 ± 5.692	17.93 ± 8.678	13	-2.308	2.160	0.019*
Bun	12.40 ± 3.247	14.60 ± 2.874	14	-2.858	2.145	0.013*
Triglycerides – TG (mg/dl)						
Muffin	73.21 ± 28.461	89.64 ± 43.388	13	-2.308	2.160	0.038*
Bun	62.13 ± 16.617	73.00 ± 14.368	14	-2.767	2.145	0.015*
Non-HDL cholesterol						
Muffin	104.14±20.508	101.64±25.267	13	0.604	2.160	0.556 NS
Bun	104.47±26.811	106.33±20.325	14	-0.636	2.145	0.535NS
Total Cholesterol to HDL ratio						
Muffin	3.36 ± 0.803	3.33 ± 0.814	13	0.250	2.160	0.806NS
Bun	3.27 ± 0.706	3.18 ± 0.442	14	0.827	2.145	0.422 NS
Triglycerides to HDL ratio						
Muffin	1.79 ± 1.040	2.17 ± 1.241	13	-2.003	2.160	0.066 NS
Bun	1.34 ± 0.401	1.51 ± 0.359	14	-2.393	2.145	0.031*
LDL to HDL ratio						
Muffin	2.09 ± 0.633	1.97 ± 0.631	13	1.058	2.160	0.309 NS
Bun	1.99 ± 0.672	1.90 ± 0.418	14	0.883	2.145	0.392 NS
LDL to VLDL ratio						
Muffin	6.74 ± 2.394	5.38 ± 2.303	13	3.379	2.160	0.005*
Bun	7.69 ± 2.255	6.40 ± 1.294	14	2.296	2.145	0.038*

Note: Values are expressed as means ± SD; Muffins: n = 14, Buns group: n =15 df: degree of freedom; *significance at 5%; NS: non-significant.

The present study results show that there is a need for in depth study to investigate the effect of millet brans on protein metabolism in body. Along with the bran enrichment, protein isolates like defatted soya, whey protein etc. can be added in the products to balance, manage or overcome this negative effect of bran on protein utilization. Also, if the decrease in protein content is solely due to the regular consumption of bran enriched products, then these products may serve as an option for the segment of population suffering from microalbuminuria and proteinuria. More research is necessary to give concrete results and understand the effect of bran fortified products' consumption and their effects on blood protein and amino acid levels.

Table 4.31. Effect of supplementation (45 days) on blood proteins levels

Total Protein (mg/dl)						
Group	**Pre**	**Post**	**df**	**t stat**	**t critical**	**p-value**
Muffin	7.18 ± 0.222	6.44 ± 0.450	13	6.039	2.160	0.000*
Bun	7.03 ± 0.313	6.61 ± 0.335	14	3.339	2.145	0.005*
Albumin (mg/dl)						
Group	**Pre**	**Post**	**df**	**t stat**	**t critical**	**p-value**
Muffin	4.32 ± 0.125	3.73 ± 0.240	13	8.578	2.160	0.000*
Bun	4.31 ± 0.110	3.69 ± 0.146	14	15.319	2.145	0.000*

Note: Values are expressed as means ± SD; Muffins: n = 14, Buns group: n =15 df: degree of freedom; *significance at 5%; NS: non-significant.

4.12.2.5 Stress profile

Serum lipid peroxidation (LP), glutathione reductase (GR) and RBC catalase were analyzed to study the effect of kodo bran muffin and foxtail bran bun on antioxidant enzyme system. Catalase and glutathione peroxidase are one of the best antioxidant enzymes defence system providing protection against oxidative stress (Fernández-Pachón *et al.* 2009). The results of supplementation study are given in Table 4.32.

4.12.2.5.1 Lipid peroxidation

After 45 days of supplementation, lipid peroxidation decreased in both buns and muffins group. Though it was not statistically significant LP (Nano moles of MDA) decreased from 3.00 ± 0.442 to 2.71 ± 0.985 and 3.40 ± 0.536 to 3.13 ± 0.894 in both muffins and buns group respectively. The decrease in lipid peroxidation in the present study could be due to the higher phenolic compounds, phytates present in kodo and foxtail bran. A previous study also suggested that phenolic compounds present in millet grains helped in inhibition of lipid oxidation (Chandrasekara and Shahidi, 2012). In lipid peroxidation mainly cell membrane is damaged due to free radicals and leads to MDA formation (Ursini and Maiorino, 2013). Reduction in MDA levels shows that lipid peroxidation is reduced, indicating less cell damage and better protection against the free radicals causing this damage. Thus, results imply that bran enriched products had good antioxidant capacity and helped in scavenging free radicals. These results are concurrent with previous study where hypercholesterolemic rats fed with barley bran showed reduction in lipid peroxidation (Abulnaja and El Rabey, 2015).

4.12.2.5.2 Glutathione reductase

Glutathione reductase catalyses conversion of glutathione disulfide (oxidised state) to glutathione (GSH) that is vital in supporting aerobic life and providing antioxidant protection, regulation of the redox status of the cells (Kehrer *et al*. 2010 and Ursini and Maiorino, 2013). Glutathione reductase significantly increased in both bun (from 377.37 ± 84.272 to 888.47 ± 454.098) and muffin groups (from 353.40 ± 48.95 to 588.85 ± 175.89) after 45 days supplementation suggesting that, when oxidative stress increased in body, glutathione reductase was actively preventing oxidative damage. Similar increase in glutathione reductase activity was noted in hypercholesterolemic rats fed with barley bran (5 %) and barley bran seed powder at 10 per cent (Abulnaja and El Rabey, 2015).

This suggests that bran enriched buns and muffins increased the antioxidant levels in serum after supplementation due to the presence of their phenolic compounds, flavonoids and phytic acid. Similarly, Fernández-Pachón *et al.* (2009) concluded that intake of polyphenols through diet can affect antioxidant enzyme system and may play important role in preventing diseases caused due to reactive oxygen species.

4.12.2.5.3 RBC catalase

Catalase helps in conversion of H_2O_2 in water (H_2O) and oxygen (O_2) thus decomposes hydrogen peroxide preventing free radical damage (Marín-García, 2014). It was evident from the results that no significant difference was noted in the muffin and bun group after 45 days of supplementation. However, slight reduction from 6.62 ± 2.589 to 5.82 ± 0.686 (µmol H2O2 consumed/min/mg) was noted in muffin group while insignificant increase *i.e.*, 6.32 ± 0.762 to 6.46 ± 1.326 (µmol H2O2 consumed/min/mg) was recorded in bun group. Similarly, Abulnaja and El Rabey (2015) also recorded increase in catalase activity in serum for hypercholesterolemic rats after feeding with barley bran (5 %) and barley bran seed powder (10 %) compared to control group. The disparity in present results could be again due to individual variations. Also, enzyme fluctuation is rapid in body and values may change instantly if person is experiencing any stress. Thus, it can be suggested that to get concrete results more controlled and long duration experiment is necessary.

Table 4.32. Effect of supplementation (45 days) on stress profile

Lipid peroxidation (Nano moles of MDA)						
Group	**Pre**	**Post**	**Df**	**t stat**	**t critical**	**p value**
Muffin	3.00 ± 0.442	2.71 ± 0.985	13	0.845	2.160	0.413^{NS}
Bun	3.40 ± 0.536	3.13 ± 0.894	14	1.046	2.145	0.313^{NS}
Glutathione reductase (units/ml)						
Muffin	353.40 ± 48.95	588.85 ± 175.89	13	-5.271	2.160	0.000^{*}
Bun	377.37 ± 84.272	888.47 ± 454.098	14	-4.335	2.145	0.001^{*}
RBC Catalase (µmol H2O2 consumed/min/mg)						
Muffin	6.62 ± 2.589	5.82 ± 0.686	13	1.236	2.160	0.238^{NS}
Bun	6.32 ± 0.762	6.46 ± 1.326	14	-0.465	2.145	0.649^{NS}

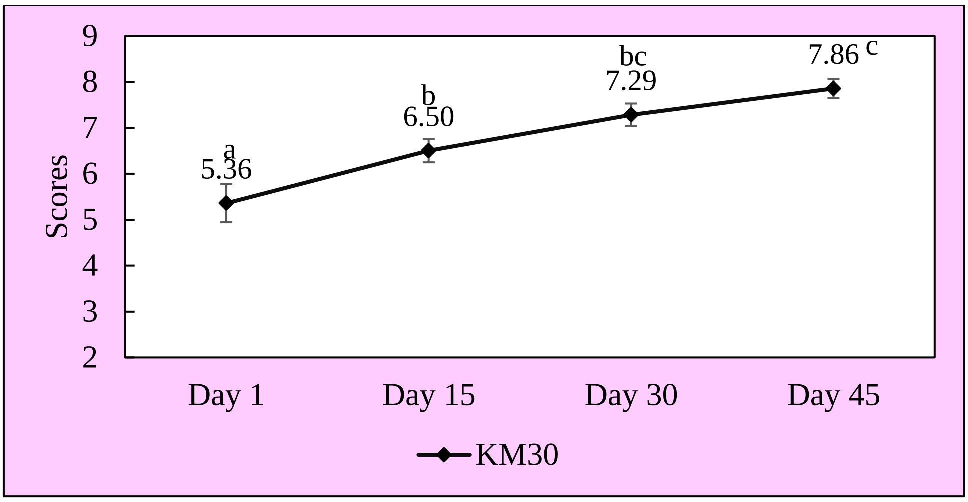
Note: Values are expressed as means ± SD; Muffins: n = 14, Buns group: n =15 df: degree of freedom; *significance at 5%; NS: non-significant.

Overall, the results for supplementation in the present study suggest that intake of polyphenol rich diet or addition of more antioxidants and phytonutrients via diet on a regular basis can play a beneficial role in preventing body from oxidative stress caused by free radicals. It is also evident from the present study that longer duration studies, with larger sample size (to minimize individual variability), controlled diet as well as conditions like exercise can help in better understanding on the consumption of bran.

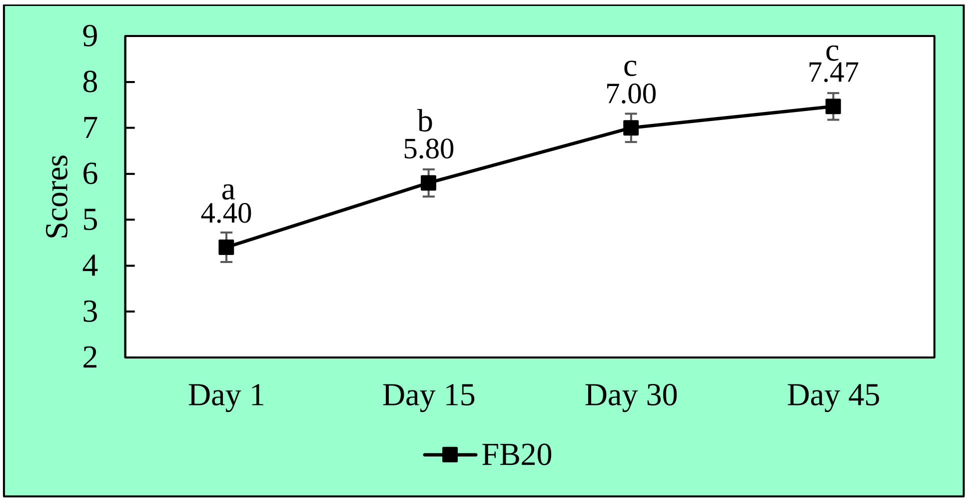
4.13 Feedback about the products by subjects during 45 days of consumption

Feedback was collected to understand subjects' response on the acceptability of the bran enriched muffins and buns after regular consumption. This was done by asking the subjects from both the groups to rate the product for overall acceptability using the score system of 9-point hedonic scale. It was observed that, the overall acceptability score of both products increased by the end of supplementation period. The results are depicted in Fig 4.17.

During the supplementation, weekly meetings were conducted to check if subjects were having any discomfort during the study and also to take feedback regarding the products provided to them. In these meeting they were also told regarding the benefits of consuming fiber through diets, to ensure that they consumed the products regularly without fail.



(a)



(b)

Fig. 4.17 Overall acceptability scores for (a) kodo bran muffin (KM30) and (b) foxtail bran bun (FB20) during 45 days supplementation

Note: Values are expressed as mean ± SE; different alphabet over each mean shows statistically significant difference at 5%; KM30: 30 per cent kodo bran muffin (n=14); FB20: 20 per cent foxtail bran bun (n=15)

The feedback sheet was filled for overall acceptability by participants on initial day (1st day), after 15th day, 30th day and on last day (45th day). Scores were given on the basis of score system of 9-point hedonic scale. Scores for kodo bran enriched muffins increased from 5.36 on initial day to 7.86 on the final (45th day). Whereas scores for buns increased from 4.40 to 7.47 on 45th day. This increase in scores indicated that gradually the participants became habitual to bran enriched muffins and buns. Comments on the score card by participants in both groups revealed that initially they had difficulty in adjusting to slight hard, grainy texture and nutty flavor of bran (foxtail and kodo). This is because of acclimatization to the soft textures of muffins and buns that are available in market. Later they developed liking towards it as they started consuming the bran enriched buns/muffins regularly, thus developing better acceptance after habitual consumption. Thus, making subjects aware about health benefits and regular consumption acceptability can be developed towards healthy snacks substitutes. Similarly, in a study positive effect on consumer acceptance in pasta with wheat bran addition up to 30 per cent was observed after providing nutritional information on fiber (Laureati *et al.* 2016).

Thus, a regular communication of healthy and nutrient dense snacks substitutes may improve the acceptability of these products over the products that lack nutrient density.

Chapter V

SUMMARY AND CONCLUSIONS

Changing life style and double burden of malnutrition challenges the food industries and scientists to develop designer products with nutrient density. In the present scenario, where consumption of bakery products has increased due to its convenience and palatability, it serves as a suitable medium for developing value-added designer snacks. Bakery products are traditionally made of refined wheat flour, sugar and fat, thus lack many essential nutrients and dietary fiber. Bran is the by-product of primary processing of cereal and other grains. It is the hard-outer layer that is discarded or used as animal feed. But, bran is nutrient dense and possess many therapeutic values. Primary processing such as dehulling, dehusking, debranning and milling for millet grains is tedious process as they are tiny in size. Hence, they yield a higher amount of bran or bran rich fractions during processing. Considering all this the present study was planned with objectives to analyze nutritional, antioxidant and phytonutrient profile and functional properties of bran. Further, the analyzed bran samples were used to developed bran enriched buns and muffins.

The study was conducted in four phases. In the first phase to obtain bran samples, minor millets viz, kodo, proso, foxtail and barnyard were dehulled using stone abrasive dehuller. Bran yield (%) from the grains ranged between 12.35 to 17.93 with kodo yielding highest amount of bran. All the grains after dehulling had significantly ($p<0.05$) reduced length, width and perimeter when compared to before dehulling. Bran samples were stabilized using different hot air oven (HT1, HT2) and microwave (MW1, MW2) heating treatments based on preliminary trials and it was noted that MW2 was the best treatment in stabilizing bran with most effective control over FFA and moisture during the storage period of 15 days. Overall, all the stabilization treatments helped in maintaining FFA below the permissible levels during storage.

In the second phase, all the microwave stabilized (MW2) bran samples were analyzed for their nutritional, antioxidant and phytonutrient profile. It was clear from the analysis that bran samples had comparable or slightly higher nutrients, antioxidants and phytonutrients than other cereal brans. When compared amongst the minor millet brans used in study, kodo bran (KSM) had highest TDF (61.52 %), TP (449.27 mg GAE/100 g), flavonoids (22.37 μg RE/ g) and phytic acid (630 mg/100 g), followed by barnyard bran (BSM). Proso bran (PSM) had a high amount of protein (13.04 g/100 g), zinc (5.59 mg/100g) and potassium (630.83 mg/100g), whereas foxtail bran (FSM) had highest fat (9.87 g/100 g), iron (65.58 mg/100 g) and calcium (94.63 mg/100g) content compared to other brans. Presence of phytate content can hinder the availability of mineral. In the present study, the phytate mineral ratios were well within critical limit for BSM in terms of calcium; PSM, FSM for iron and PSM, BSM and FSM for zinc. Further, the physicochemical properties of bran revealed that, bran had oil and water absorption capacity ranging between 162.62 – 258.80 g/100 g, 246.47 – 298.42 g/100 g respectively, but showed no foaming ability. Nutritional, antioxidant profile of bran indicate that they can be used for improving therapeutic benefits of products also their water and oil absorption capacity imply that they have potential of being exploited as functional ingredient in processing industry.

In the third phase of the study, brans were incorporated in different concentration (0, 10, 15, 20, 25 and 30 %) into refined wheat flour. Further studies on physicochemical properties showed, WAC of value-added flours increased as the bran concentration in flour increased, but OAC, WSI and FC decreased. Both buns and muffins developed using minor millet brans showed a reduction in weight and increase in height after baking. But, as the bran percentage increased in buns and muffins, there was an insignificant increase in baked weight and decrease in height. The baking loss rate (%) lowered with the addition of bran, but it was insignificant in all bran enriched muffins and buns. First level sensory evaluation of buns showed that 20 per cent bran blending was preferred amongst all the variations irrespective of the source of bran. In the second level of sensory evaluation, among all 20 per cent bran enriched buns, foxtail bran bun was selected by the semi-trained panel members over Kodo, proso and barnyard bran buns. Concerning muffins in first level sensory, 30 per cent bran enrichment was found palatable by the panel members. Further among all muffins with 30 per cent bran, kodo muffin was preferred over foxtail, proso and barnyard bran muffins. Nutritional profile of designer kodo bran muffin (KM30) and foxtail bran bun (FM20) revealed that they had higher nutrients, antioxidant and phytonutrients compared to their respective control. Amount of TDF (%) increased from 0.74 to 2.73 (FM20) and 0.75 to 2.79 (KM30). Under shelf life studies moisture, water activity, microbial count and sensory evaluation was conducted and the products showed shelf life of 3 (buns) and 7 (muffins) days. Moisture, water activity and microbial count increased during storage, but sensory scores decreased.

In the fourth phase supplementation studies for FM20 and KM30 were conducted on 2 groups with adolescent participants (n=14 and n=15 respectively). Results of supplementation study indicated, that bran enriched products for 45 days did not alter the normal blood glucose, lipid and stress (lipid peroxidation, RBC catalase, glutathione reductase) profile of healthy adolescent subjects. Hence, it is opined that replacing traditional refine flour based bakery products with designer products is beneficial for adolescent health.

However, in order to the establish the impact of bran enriched products consumption on each blood parameter, longer duration and controlled studies are necessary.

Practical implications/highlights of the study

- Minor millet bran obtained as by-products during processing had good amount of proximate (protein (5.678 to 13.037 g/100g), fat (5.283 to 9.870 g/100g), ash (6.895 to 12.151 g/100g) and crude fiber (29.337 to 32.519 g/100g)), mineral, antioxidants and anti-nutrient content.
- Kodo bran had highest amount of dietary fiber, total phenol, flavonoids, phytic acid and antioxidant capacity.
- Reformulation of buns with foxtail bran (20 %) and muffins with kodo bran (30 %) significantly enhanced nutrient profile, antioxidant capacity, minerals and dietary fibre.
- Bran enriched buns and muffins had medium glycemic index and glycemic load which are good substitutes for regular buns and muffins available in the market.
- Inclusion of bran to a certain percentage did not affect the quality of the buns and muffins
- Minor millet brans can be used as a promising functional ingredient in bakery and food processing industry.

Future line of work

- Complete profiling of minor millet brans for their fatty acid and amino acids can be taken up.
- Bioavailability studies can be conducted to understand availability of nutrients.
- Bioprocessing or micronisation processes of bran can be tried to increase the percentage of bran that can be incorporated in products.

- Various other value-added products can be designed by incorporating brans.
- Long duration, controlled studies on larger section of population can be performed to understand and generate concrete evidence of therapeutic benefits of minor millet bran supplementation.

LITERATURE CITED

Abdul-Hamid, A., Sulaiman, R. R. R., Osman, A and Saari, N. 2007. Preliminary study of the chemical composition of rice milling fractions stabilized by microwave heating. *Journal of Food Composition and Analysis*. 20: 627–637.

Abdulwaliyu, I., Arekemase, S.O., Adudu, J.A., Batari, M.L., Egbule, M.N and Reuben Okoduwa, S.I. 2019. Investigation of the medicinal significance of phytic acid as an indispensable anti-nutrient in diseases. *Clinical Nutrition Experimental.* 28: 42 – 61. Doi: 10.1016/j.yclnex.2019.10.002

Abulnaja, K.O and El Rabey, H.A. 2015. The efficiency of barley (*Hordeum vulgare*) bran in ameliorating blood and treating fatty heart and liver of male rats. *Evidence-Based Complementary and Alternative Medicine*. pp13. Doi: https://doi.org/10.1155/2015/740716

Afaghi, A., Ghanei, L and Ziaee, A. 2013. Effect of low glycemic load diet with and without wheat bran on glucose control in gestational diabetes mellitus: A randomized trial. *Indian Journal of Endocrinology and Metabolism*. 17: 689–692. Doi: https://www.ijem.in/text.asp?2013/17/4/689/113762

Ahmad, F., Pasha, I., Saeed, M and Asgher, M. 2018. Biochemical profiling of Pakistani sorghum and millet varieties with special reference to anthocyanins and condensed tannins. *International Journal of Food Properties*. 21 (1): 1586-1597.

Ahmad, M., Baba, W.N., Wani, T.A., Gani, A., Gani, A., Shah, U., Wani, S.M and Masoodi, F.A. 2015. Effect of green tea powder on thermal, rheological and functional properties of wheat flour and physical, nutraceutical and sensory analysis of cookies. *Journal of Food Science and Technology.* 52 (9): 5799–5807.

Ajila, C.M., Leelavathi, K and Prasada, U.J.S.R. 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science*. 48: 319–326.

Akanbi, T.O., Timilsena, Y and Dhital, S. 2019. Bioactives from millet: properties and effects of processing on bioavailability. In J. Wang, B. Sun and R. Tsao (eds.)- *Bioactive Factors and Processing Technology for Cereal Foods.* pp. 171–183. Springer, Singapore. Doi: https://doi.org/10.1007/978-981-13-6167-8_10

Akbarpour, S., Khalili, D., Zeraati, H., Mansournia, M. A., Ramezankhani, A., Pishkuhi, M. A., Gooran, S. R and Fotouhi, A. 2019. Relationship between lifestyle pattern and blood pressure – Iranian national survey. *Scientific Reports*. 9:15194. Doi: https://doi.org/10.1038/s41598-019-51309-3

Akhter, M., Afzal, N., Haider, Z and Raza, M.A. 2015. Inactivation of lipase enzyme by using chemicals to maximize rice bran shelf life and its edible oil recovery. *Integrative Food, Nutrition and Metabolism.* 2 (2): 166–170.

Althwab, S. A. 2013. The effect of wheat bran muffin on weight, blood glucose, and lipid. *Master's Thesis,* University of Wisconsin-Stout, Graduate School.

Amadou, I. Amza, T., Shi, Y-H and Le, G-W. 2011. Chemical analysis and antioxidant properties of foxtail millet bran extracts. *Songklanakarin Journal Science and Technology*. 33 (5): 509–515.

Amadou, I., Gounga, M.E and Le, G. 2013. Millets: Nutritional composition, some health benefits and processing – A Review. *Emirates Journal of Food and Agriculture*. 25 (7): 501-508.

AOAC. 1980. Official methods of analysis 13th ed. Association of Official Analytical chemists, Washington, D.C.

AOAC. 1990. Official method 956.01 Potassium and/or Sodium in Plants Atomic Absorption Spectrophotometry. 15th ed, *Association of Official Analytical Chemists International.* Chapter 3. pp 47.

AOAC. 1990. Official method 975.03 Metals in Plants Atomic Absorption Spectrophotometry. 15th ed, *Association of Official Analytical Chemists International.* Chapter 3. pp 42.

AOAC. 2006. Official methods of analysis Total Carbohydrates, crude 'by difference' Calculation. 100 percent minus percent (CP + Ash + Crude Fat + M) - item 85. *Association of Analytical Communities,* Gaithersburg, MD, 17th ed. NFNAP; PROX.

AOAC. 2007. Official method 922.06. Crude fat. *Association of Official Analytical Chemists International*. 18th ed, Chapter 32 (Horwitz, W., ed.), p. 5. Gaithersburg, MD.

AOAC. 2007. Official method 962.09 Fibre (Crude) in Animal feed and Pet Food. *Association of Official Analytical Chemists International*. 18th ed, Chapter 4 (Horwitz, W., ed.), p. 44. Gaithersburg, MD.

AOAC. 2010. Official method 940.28. Fatty Acid (Free) in crude and refined oils. *Association of Official Analytical Chemists International*. 18th ed, 4th Revision Chapter 41 (Horwitz, W., ed.), p.12. Gaithersburg, MD.

AOAC. 2010. Official method 954.01. Protein (crude) in Animal Feed. *Association of Official Analytical Chemists International*. 18th ed, 4th Revision Chapter 4 (Horwitz, W., ed.), p.24. Gaithersburg, MD.

AOAC. 2010. Official Methods of Analysis of AOAC International, AOAC 985.29. Fiber (Total Dietary) 18th ed, 4th Revision Chapter 32 (Horwitz, W., ed.), pp.5-13. Gaithersburg, MD

Arendt, E.K and Zannini, E. 2013a. Rice. In Arendt, E. K and Zannini, E. (eds.)- *Cereal Grains for the Food and Beverage Industries.* Woodhead Publishing Limited, UK. Pp. 114 – 154. Doi: 10.1533/9780857098924.114.

Arendt, E.K and Zannini, E. 2013b. Millet. Cereal Grains for the Food and Beverage Industries. In Arendt, E.K and Zannini, E. (eds.) - *Cereal Grains for the Food and Beverage Industries.* Woodhead Publishing Series in Food Science, Technology and Nutrition. 312–350. Doi: 10.1533/9780857098924.312.

Arora, A and Saini, C. S. 2016. Development of bun from wheat flour fortified with de-oiled maize germ. *Cogent Food and Agriculture.* 2: 1183252. Doi: http://dx.doi.org/10.1080/23311932.2016.1183252.

Aston, L., Gambell, J., Lee, D., Bryant, S.P and Jebb, S.A. 2008. Determination of the glycaemic index of various staple carbohydrate-rich foods in the UK diet. *European Journal of Clinical Nutrition.* 62: 279–285. Doi: https://doi.org/10.1038/sj.ejcn.1602723

Awika, J.M., Rooney, L.W and Waniska, R.D. 2004. Properties of 3-Deoxyanthocyanins from Sorghum. *Journal of Agriculture and Food Chemistry.* 52: 4388–4394.

Ayala-Soto, F.E., Serna-Saldívar, S.O., Welti-Chanes, J and Gutierrez-Uribe, J.A. 2015. Phenolic compounds, antioxidant capacity and gelling properties of glucoarabinoxylans from three types of sorghum brans. *Journal of Cereal Science.* 65: 277–284.

Ayub, M., Wahab, S and Durrani, Y. 2003. Effect of water activity (aw) moisture content and total microbial count on the overall quality of bread. *International Journal of Agriculture and Biology*. 5 (3): 274–278.

Azad, M.O.K., Jeong, D.I., Adnan, M., Salitxay, T., Heo, J.W., Naznin, M.T., Lim, J.D., Cho, D.H., Park, B.J and Park, C.H. 2019. Effect of different processing methods on the accumulation of the phenolic compounds and antioxidant profile of broomcorn millet (*Panicum miliaceum L.*) flour. *Foods*. 8: 230. Doi: 10.3390/foods8070230.

Bagchi, T.B., Adak, T and Chattopadhyay K. 2014. Process standardization for rice bran stabilization and its' nutritive value. *Journal of Crop and Weed*. 10 (2): 303-307.

Bagheri, R and Seyedein, S.M. 2011. The effect of adding rice bran fibre on wheat dough performance and bread quality. *World Applied Sciences Journal*. 14 (Special Issue of Food and Environment): 121-125.

Bartalné-Berceli M., Izsó E., Gergely S and Salgó A. 2018. Development and application of novel additives in bread-making. *Czech Journal of Food Science.* 36 (6): 470–475. Doi: https://doi.org/10.17221/380/2017-CJFS.

Begum, R., Ahmed, S., Hakim, M.L and Sen, J. 2018. Comparative Study among Composite Breads Incorporated with Full Fatted and Defatted Rice Bran. *Journal of Environmental Science and Natural Resources.* 11 (1&2): 43–52.

Benzie, I.F.F and Strain, J.J. 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology.* 299: 15–27.

Bergmeyer, H.U., Horder, M and Rej, R. 1986. Approved recommendation on IFCC (The International Federation of Clinical Chemistry) methods for the measurement of catalytical concentration of enzyme, Part 3 IFCC. 24: 481–489.

Beto, J.A. 2015. The Role of Calcium in Human Aging. *Clinical Nutrition Research.* 4: 1-8. Doi: http://dx.doi.org/10.7762/cnr.2015.4.1.1

Bhat, V.B., DayakarRao, B and Tonapi, V.A. 2018. *The Story of Millets*. Karnataka State Department of Agriculture, Bengaluru, India with ICAR-Indian Institute of Millets Research, Hyderabad, India. 1-109.

Bhavya, S.N and Prakash, J. 2018. Nutritional and sensory quality of buns enriched with soy fiber (*Okara*). *Journal of Engineering and Processing Management*. 10 (2): 23-31. Doi: https://doi.org/10.7251/JEPM181002023B

Bhise, S and Kaur, A. 2014. Baking quality sensory properties and shelf life of bread with polyols. *Journal of Food Science and Technology.* 51 (9): 2054–2061.

Bhosale, S and Vijayalakshmi, D. 2015. Processing and Nutritional Composition of Rice Bran. *Current Research in Nutrition and Food Science Journal.* 3 (1): 74-80.

Bijalwan, V., Ali, U., Kesarwani, A.K., Yadav, K and Mazumder, K. 2016. Hydroxycinnamic acid bound arabinoxylans from millet brans-structural features and antioxidant activity. *International Journal of Biological Macromolecules*. 88: 296–305.

Bindra, G.S., Gibson, R.S and Thompson, L.U. 1986. [Phytate]×[calcium]/[zinc] ratios in Asian immigrant lacto-ovo vegetarian diets and their relationship to zinc nutriture. *Nutrition Research.* 6: 475-483.

Bisoi, P.C., Sahoo, G., Mishra, S.K., Das, C and Das, K. L. 2012. Hypoglycemic effects of insoluble fiber rich fraction of different cereals and millets. *Journal of Food Processing and Technology*. 3 (7): 1-11. Doi:10.4172/2157-7110.1000191

Blois, M.S. 1958. Antioxidant determination by the use of a stable free radical. *Nature*. 26: 1199–1200.

Bora, P., Das, P., Bhattacharyya, R and Saikia, A. 2018. Effect of processing on the phytochemical content and antioxidant capacity of proso millet (*Panicum miliaceum L.*) milled fractions. *International Journal of Chemical Studies.* 6 (4): 18-22.

Brouns, F., Hemery, Y., Price, R and Anson, N.M. 2012. Wheat aleurone: separation, composition, health aspects, and potential food use. *Critical Reviews in Food Science and Nutrition.* 52: 553–568.

Bunde, M.C., Osundahunsi F.O and Akinoso, R. 2010. Supplementation of biscuit using rice bran and soybean flour. *African Journal of Food Agriculture Nutrition and Development.* 10 (9): 4047–4059.

Butt, M.S., Qamar, M.I., Anjum, F.M., Aziz, A and Randhawa, M.A. 2004. Development of minerals-enriched brown flour by utilizing wheat milling by-products. *Nutrition and Food Science*. 34 (4): 161–165.

Caleja, C., Barros, L., Antonio, A. L., Oliveira, M. B. P. P and Ferreira, I. C. F. R. 2017. A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits. *Food Chemistry*. 216: 342 – 346. Doi: 10.1016/j.foodchem.2016.08.075

Chalamacharla, R.B., Harsha, K., Sheik, K.B and Viswanatha, C.K. 2018. Wheat Bran-Composition and Nutritional Quality: A Review. *Advances in Biotechnology and Microbiology*. 9 (1): 555754. Doi: 10.19080/AIBM.2018.09.555754

Chandi, G.K and Annor, G.A. 2016. Millet Minor: Overview. In Wrigley, C. W. Corke, H. Seetharaman, K and Faubion, J. (eds.) – *Encyclopedia of Food Grains. 2nd ed. Volume 1: The World of Food Grains*. Elsevier Ltd. 199-208.

Chandra, S., Singh, S and Kumari, D. 2015. Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology.* 52 (6): 3681–3688. Doi: 10.1007/s13197-014-1427-2.

Chandrasekara, A and Shahidi, F. 2011. Bioactivities and antiradical properties of millet grains and hulls. *Journal of Agricultural and Food Chemistry*. 59: 9563–9571.

Chandrasekara, A and Shahidi, F. 2012. Antioxidant phenolics of millet control lipid peroxidation in human LDL cholesterol and food systems. *Journal of* American *Oil Chemists Society*. 89: 275–285. Doi: 10.1007/s11746-011-1918-5

Chaplin, M.F. 2003. Fiber and water binding. *Proceedings of Nutrition Society*. 62: 223–227. Doi:10.1079/PNS2002203

Cho, S.S., Qi, L., Fahey, G.C., Jr and Klurfeld, D.M. 2013. Consumption of cereal fiber, mixtures of whole grains and bran, and whole grains and risk reduction in type 2 diabetes, obesity, and cardiovascular disease. *The American Journal of Clinical Nutrition.* 98 (2): 594–619. Doi: https://doi.org/10.3945/ajcn.113.067629

Choi, Y.S., Choi, J.H., Han, D.J., Kim, H.Y., Lee, M.A., Kim, H.W., Jeong, J.Y and Kim, C.J. 2011. Effects of rice bran fibre on heat-induced gel prepared with pork salt-soluble meat proteins in model system. *Meat Science.* 88: 59–66.

Clavero, M.R., Brackett, R., Beuchat, L and Doyle, M. 2000. Influence of water activity and storage conditions on survival and growth of proteolytic Clostridium botulinum in peanut spread. *Food Microbiology.* 17 (1): 53–61. Doi: 10.1006/fmic.1999.0292

Coda, R., Katina, K and Rizzello, C. G. 2015. Bran bioprocessing for enhanced functional properties. *Current Opinion in Food Science*. 1: 50–55

Cohen, G., Dembiec, D and Marcus, J. 1970. Measurement of catalase in tissue extract. *Analytical Biochemistry.* 34: 30-38.

Curti, E., Carini, E., Bonacini, G., Tribuzio, G and Vittadini, E. 2013. Effect of the addition of bran fractions on bread properties. *Journal of Cereal Science.* 57: 325–332.

Dahlberg, J.A., Wilson, J.P and Snyder, T. 2004. Sorghum and Pearl Millet: Health Foods and Industrial Products in Developed Countries. CFC and ICRISAT. 2004. Alternative Uses of Sorghum and Pearl Millet in Asia: proceedings of the Expert Meeting, ICRISAT, Patancheru, Andhra Pradesh, India, 1-4 July 2003. CFC Technical Paper No. 34. P.O. Box 74656, 1070 BR Amsterdam, The Netherlands: Common Fund for Commodities; and Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. pp 364 pp.

Daou, C and Zhang, H. 2014. Functional and physiological properties of total, soluble, and insoluble dietary fibres derived from defatted rice bran. *Journal of Food Science and Technology.* 51 (12): 3878-3885. Doi: 10.1007/s13197-013-0925-y.

Dar, B.N and Sharma, S. 2011. Total phenolic content of cereal brans using conventional and microwave assisted extraction. *American Journal of Food Technology*. 6 (12): 1045-1053. Doi: 10.3923/ajft.2011.1045.1053

Dar, B.N., Sharma, S and Nayik, G.A. 2016. Effect of storage period on physiochemical, total phenolic content and antioxidant properties of bran enriched snacks. *Food Measurement.* 10: 755–761. Doi: https://doi.org/10.1007/s11694-016-9360-x

Dar, B.N., Sharma, S., Singh, B and Kaur, G. 2014. Quality Assessment and Physicochemical Characteristics of Bran Enriched *Chapattis. International Journal of Food Science.* 1–6. Doi: http://dx.doi.org/10.1155/2014/689729.

Daud, N.S.M., Zaidel, D.N.A., Song L.K., Jusoh Y.M.M., Muhamad I.I and Ya'akob H. 2017. Microwave-assisted stabilization and storage stability study of rice bran oil from different varieties. *Chemical Engineering Transactions*. 56: 1285-1290.

DayakarRao, B., Ananthan, R., Hariprasanna, K., Bhatt, K.V., Rajeswari, Sharma, S and Tonapi, V.A. 2018. *Nutritional and Health Benefits of Nutri Cereals.* Nutri hub TBI, ICAR-Indian Institute of Millets Research (IIMR) Rajendranagar, Hyderabad. pp 96.

de Munter, J.S.L., Hu, F.B., Spiegelman, D., Franz, M and van Dam, R.M. 2007. Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *PLoS Medicine.* 4 (8): e261. Doi: 10.1371/journal.pmed.0040261.

de Souza, C.B., Lima, G.P.P., Borges, C.V., Dias, L.C.G.D., Spoto, M.H.F., Castro, G. R., Corrêa, C.R and Minatel, I.O. 2019. Development of a functional rice bran cookie rich in γ-oryzanol. *Journal of Food Measurement and Characterization.* 13: 1070–1077. Doi: https://doi.org/10.1007/s11694-018-00022-2

Deshpande, S.S., Mohapatra, D., Tripathi, M.K and Sadvatha, R.H. 2015. Kodo Millet-Nutritional Value and Utilization in Indian Foods. *Journal of Grain Processing and Storage*. 2 (2): 16-23.

Devi, P.B., Vijayabharathi, R. Sathyabama, S. Malleshi, N.G and Priyadarisini, V.B. 2014. Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. *Journal of Food Science and Technology*. 51 (6): 1021–1040.

Devisetti, R., Yadahally, S.N and Bhattacharya, S. 2014. Nutrients and antinutrients in foxtail and proso millet milled fractions: Evaluation of their flour functionality. *LWT - Food Science and Technology*. 59: 889–895.

Devittori, C., Gumy, D., Kusy, A., Colarow, L., Bertoli, C and Lambelet, P. 2000. Supercritical fluid extraction of oil from millet bran. *Journal of American Oil Chemists Society*. 77 (6): 573-579.

Dhankhar, J. Vashistha, N and Sharma, A. 2019. Development of biscuits by partial substitution of refined wheat flour with chickpea flour and date powder. *Journal of Microbiology Biotechnology and Food Sciences*. 8 (4): 1093-1097. Doi: 10.15414/jmbfs.2019.8.4.1093-1097.

Dharmaraj, U., Sathyendra Rao, B.V., Sakhare, S.D and Inamdar, A.A. 2016. Preparation of semolina from foxtail millet (*Setaria italica*) and evaluation of its quality characteristics. *Journal of Cereal Science*. 68: 1–7. Doi: 10.1016/j.jcs.2015.11.003.

Dhillon, P. K and Tanwar, B. 2018. Muffins incorporated with multiple blend functional ingredients: development, sensory evaluation, proximate composition and total antioxidant activity. *Journal of Agricultural Engineering and Food Technology*. 5 (3): 122–126.

Dhingra, D., Chopra, S and Rai, D.R. 2012. Stabilization of raw rice bran using ohmic heating. *Agricultural Research*. 1 (4): 392–398.

Directorate of Economics and Statistics. 2018. Third Advance Estimates of Production of Food grains for 2017-18.

Directorate of Millets Development. 2017a. Foxtail millet (*Setaria italica* L.). 01 May 2017. http://millets.dacfw.nic.in/POP%20Foxtail.html

Directorate of Millets Development. 2017b. Proso millet (*Panicum miliaceum* L.). 01 May 2017. http://millets.dacfw.nic.in/POP%20Proso.html

Directorate of Millets Development. 2017c. Kodo millet (*Paspalum scrobiculatum L.*). 01 May 2017. http://millets.dacfw.nic.in/POP%20Kodo.html

Directorate of Millets Development. 2017d. Barnyard millet (*Echinochloa frumentacea* L.). 01 May 2017. http://millets.dacfw.nic.in/POP%20Barnyard.html

Dodd, H., Williams, S., Brown, S and Venn, B. 2011. Calculating meal glycemic index by using measured and published food values compared with directly measured meal glycemic index. *American Journal of Clinical Nutrition*. 94: 992 – 996.

Dong, J., Wang, L., Lü, J., Zhu, Y and Shen, R. 2019. Structural, antioxidant and adsorption properties of dietary fiber from foxtail millet (*Setaria italica*) bran.

Journal of the Science of Food and Agriculture. 3886–3894. Doi: 10.1002/jsfa.9611.

Doulia, D. Katsinis, G and Rigas, F. 2006. Prediction of the mould- free shelf life of muffins. *International Journal of Food Properties*. 9 (4): 637-650. Doi: 10.1080/10942910600853824.

Durairaj, M., Gurumurthy, G., Nachimuthu, V., Muniappan, K and Balasubramanian, S. 2019. Dehulled small millets: The promising nutricereals for improving the nutrition of children. Maternal and Child Nutrition. 15 (S3): e12791. Doi: https://doi.org/10.1111/mcn.12791

Dykes, L and Rooney, L.W. 2007. Phenolic compounds in cereal grain and their health benefits. *Cereal Foods World*. 105–111.

Edrisi, F., Salehi, M., Ahmadi, A., Fararoei, M., Rusta, F and Mahmodianfard, S. 2017. Effects of supplementation with rice husk powder and rice bran on inflammatory factors in overweight and obese adults following an energy-restricted diet: a randomized controlled trial. *European Journal of Nutrition*. Doi: 10.1007/s00394-017-1555-3.

Egbedike, C.N., Ozo, N.O., Ikegwu, O.J., Odo, M.O and Okorie, P.A. 2016. Effect of rice bran substitution on the physicochemical properties of water yam flour. *Asian Journal of Agriculture and Food Sciences.* 4 (5): 246–257.

Eleazu C.O. 2016. The concept of low glycemic index and glycemic load foods as panacea for type 2 diabetes mellitus; Prospects, challenges and solutions. *African Health Science*. 16 (2): 468-479. Doi: http://dx.doi.org/10.4314/ahs.v16i2.15

El-Sharnouby, G.A., Aleid, S.M and Al-Otaibi, M.M. 2012. Nutritional quality of biscuit supplemented with wheat bran and date palm fruits (*Phoenix dactylifera* L.). *Food and Nutrition Sciences*. 3: 322–328. Doi: http://dx.doi.org/10.4236/fns.2012.33047

Ertas, N. 2015. Effect of wheat bran stabilization methods on nutritional and physico-mechanical characteristics of cookies. *Journal of Food Quality*. 38: 184–191.

Espinosa-Mendoza, R.E., Solorza-Feria, J., Arenas-Ocampo, M.L., Camacho-Diaz, B.H., Villar-Martinez, A.A.D., Vanegas-Espinoza, P.E and Jimenez-Aparicio, A.R. 2012. Morpho structural characterization of rice grain (*Oryza sativa L.*) variety

Morelos A-98 during filling stages. *The Scientific World Journal.* 1-9. Doi:10.1100/2012/940293.

Esposito, F., Arlotti, G., Bonifati, A.M., Napolitano, A., Vitale, D and Fogliano, V. 2005. Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International*. 38: 1167–1173.

Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, and National Heart, Lung, and Blood Institute. 2011. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: Summary report. *Pediatrics*. 128 (Suppl 5): S213–S256. Doi: https://doi.org/10.1542/peds.2009-2107C

FAO. 2018. Dietary Assessment: A resource guide to method selection and application in low resource settings. Food and Agricultural Organisation, Rome.

FAO/IZiNCG. 2018. FAO/INFOODS/IZiNCG Global Food Composition Database for Phytate Version 1.0 - PhyFoodComp 1.0. Rome, Italy.

Fardet, A. 2010. New hypotheses for the health protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews*. 23: 65–134.

FDA. 2008. *Health claims: fruits, vegetables, and grain products that contain fiber, particularly soluble fiber, and risk of coronary heart disease*. In Code of Federal Regulations; Food and Drug Administration: Silver Spring, MD, USA, Volume 2.

Fei, H., Lu, Z., Wenlong, D and Aike, L. 2018. Effect of roasting on phenolics content and antioxidant activity of proso millet. *International Journal of Food Engineering.* 4 (2): 110-116.

Fernández-Pachón, M.S., Berná, G., Otaolaurruchi, E., Troncoso, A.M., Martín, F and García-Parrilla, M.C. 2009. Changes in antioxidant endogenous enzymes (activity and gene expression levels) after repeated red wine intake. *Journal of Agricultural and Food Chemistry*. 57 (15): 6578–6583. Doi:10.1021/jf901863w

Fleischman, E. F., Kowalski, R. J., Morris, C. F., Nguyen, T., Li, C., Ganjyal, G and Ross, C. F. 2016. Physical, textural, and antioxidant properties of extruded waxy wheat flour snack supplemented with several varieties of bran. *Journal of Food Science.* 81 (11): 2726-2733. Doi: 10.1111/1750-3841.13511

Fontana, A.J. 2000. Understanding the importance of water activity in food. *Cereal Foods World*. 45 (1): 7-10.

Frazier, W.C., Westhoff, D.C and Vanitha, N.M. 2014. *Food Microbiology*. 5th edition. McGraw Hill Education (India) Private Limited, New Delhi. pp: 21-28.

Friedewald, W.T., Levy, R.I and Fredrickson, D.S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry.* 18 (6): 499–502.

FSANZ. 2016. Food Standards Australia New Zealand (FSANZ). Compendium of Microbiological Criteria for Food. https://www.foodstandards.gov.au/publications/Documents/Compedium%20of%20Microbiological%20Criteria/Compendium%20of%20Microbiological%20Criteria.pdf. Accessed 4 June 2020.

Fuhrman, J. 2018. The hidden dangers of fast and processed food. *American Journal of Lifestyle Medicine*. 12 (5): 375-381.

Gaitan, E., Lindsay, H.R., Reichert, D.R., Ingbarf, S.H., Cooksey, R.C., Legan, J., Meydrech, E.F., Hill, J and Kubota, K. 1989. Antithyroid and goitrogenic effects of millet: role of c-glycosylflavones. *Journal of Clinical Endocrinology and Metabolism*. 68 (4): 707-714.

Gajula, H., Alavi, S., Adhikari, K and Herald, T. 2008. Precooked bran-enriched wheat flour using extrusion: dietary fiber profile and sensory characteristics. *Journal of Food Science*. 73 (4): 173-179.

Ganesan, K and Xu, B. 2017. Polyphenol-Rich lentils and their health promoting effects. *International Journal of Molecular Sciences*. 18 (11): 2390. Doi: 10.3390/ijms18112390

Gautam, A.K., Dinesh, K., Shivay, Y.S and Mishra, B.N. 2008. Influence of nitrogen levels and plant spacing on growth, productivity and quality of two inbred varieties and a hybrid of aromatic rice. *Archives of Agronomy and Soil Science*. 54 (5): 515–532. Doi: 10.1080/03650340802283470

Geleijnse, J.M., Witteman, J.C., Stijnen, T., Kloos, M.W., Hofman, A., Grobbee, D.E. 2007. Sodium and potassium intake and risk of cardiovascular events and all-cause mortality: The Rotterdam Study. *European Journal of Epidemiology*. 22 (11): 763–770. Doi: https://doi.org/10.1007/s10654-007-9186-2

Gerhardt, A.L and Gallo, N.B. 1998. Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. *Journal of Nutrition*. 128: 865–869.

Giannini, C., Santoro, N., Caprio, S., Kim, G., Lartaud, D., Shaw, M., Pierpont, B and Weiss, R. 2011. The triglyceride-to-HDL cholesterol ratio: association with insulin resistance in obese youths of different ethnic backgrounds. *Diabetes Care*. 34: 1869-1874.

Gibson, R.S., Bailey, K.B., Gibbs, M and Ferguson, E.L. 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin.* 31: S134-S146.

Goswami, D., Gupta, R.K., Mridula, D., Sharma, M and Tyagi, S.K. 2015. Barnyard millet-based muffins: Physical, textural and sensory properties. *LWT - Food Science and Technology*. 64 (1): 374–380. Doi: https://doi.org/10.1016/j.lwt.2015.05.060.

Goudar, G and Sathisha, G.J. 2016. Effect of processing on ferulic acid content in foxtail millet (*Setaria italica*) grain cultivars evaluated by HPTLC. *Oriental Journal of Chemistry*. 32 (4): 2251-2258.

Graf, E. 1990. Antioxidant functions of phytic acid. *Free Radical Biology and Medicine.* 8 (1): 61–69. Doi:10.1016/0891-5849(90)90146-a

Green, D.M., Ropper, A.H and Kronmal, R.A. 2002. Serum potassium level and dietary potassium intake as risk factors for stroke. *Neurology.* 59: 314–20.

Gul, H., Kart, F.M., Gul, M and Akpinar, M.G. 2017. Bakery products consumption and consumers' awareness in urban areas of Isparta city, Turkey. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*. 17 (2): 137 – 146.

Gul, K., Yousuf, B., Singh, A.K., Singh, P and Wani, A.A. 2015. Rice bran: Nutritional values and its emerging potential for development of functional food – A review. *Bioactive Carbohydrates and Dietary Fibre* 6: 24–30. Doi: http://dx.doi.org/10.1016/j.bcdf.2015.06.002.

Gulia, S.K., Wilson, J.P., Carter, J and Singh, B.P. 2007. Progress in grain pearl millet research and market development. In Janik, J., Whipkey, A (eds). *Issues in new crops and new uses.* ASHS Press, Alexandria, VA. 196–203.

Guo, X., Sha, X., Rahman, E., Wang, Y., Ji, B., Wu, W and Zhou, F. 2018. Antioxidant capacity and amino acid profile of millet bran wine and the synergistic interaction between major polyphenols. *Journal of Food Science and Technology*. 55 (3): 1010–1020.

Gupta, R.K., Gangoliya, S.S and Singh, N.K. 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*. 52(2): 676–684. Doi: https://doi.org/10.1007/s13197-013-0978-y

Habiyaremye, C., Matanguihan, J.B., Guedes, J.D., Ganjyal, G.M., Whiteman, M.R., Kidwell, K.K and Murphy, K.M. 2017. Proso millet (*Panicum miliaceum L.*) and its potential for cultivation in the pacific northwest, U.S.: A Review. *Frontiers in Plant Science*. 7: 1961. Doi: 10.3389/fpls.2016.01961

Heinio, R.L., Noort, M.W.J., Katina, K., Alam, S.A., Sozer, N., de Kock, H.L., Hersleth, M and Poutanen, K. 2016. Sensory characteristics of wholegrain and bran-rich cereal foods – A review. *Trends in Food Science & Technology*. 47: 25 – 38. Doi: http://dx.doi.org/10.1016/j.tifs.2015.11.002

Heo, Y., Kim, M., Lee, J and Moon, B. 2019. Muffins enriched with dietary fiber from kimchi by-product: Baking properties, physical–chemical properties, and consumer acceptance. *Food Science and Nutrition.* 7: 1778–1785.

Herald, T.J., Gadgil, P and Tilley, M. 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture*. 92 (11): 2326-31.

Heuzé, V., Tran, G., Sauvant, D., Bastianelli, D and Lebas F. 2015. Foxtail millet (Setaria italica), grain. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/725. Last updated on May 11, 2015. 14:34.

Hines L. R. 2007. Development of specialty breads as nutraceutical products. *Master of Science Thesis*. Texas A&M University.

Ho, L.H., Abdul, A.N.A., Bhat, R and Azahari, B. 2014. Storage studies of bread prepared by incorporation of the banana pseudo-stem flour and the composite breads containing hydrocolloids. *CyTA - Journal of Food*. 12 (2): 141-149. Doi: 10.1080/19476337.2013.806597

Hussein, A.M.S and Ibrahim, G.E. 2019. Effects of various brans on quality and volatile compounds of bread. *Foods and Raw Materials*. 7 (1): 42–50. Doi: http://doi.org/10.21603/2308-4057-2019-1-42-50.

ICMR. 2020. Nutrient requirements for Indians. A report of the expert group, 2020. Indian Council of Medical Research-National Institute of Nutrition, New Delhi, 2020. https://www.nin.res.in/nutrition2020/RDA_short_report.pdf

Ikuomola, D.S., Otutu, O.L and Oluniran, D.D. 2017. Quality assessment of cookies produced from wheat flour and malted barley (*Hordeum vulgare*) bran blends. *Cogent Food and Agriculture.* 3: 1293471. Doi: http://dx.doi.org/10.1080/23311932.2017.1293471

IS 1155:1968. 2010. Specification for Wheat Atta / *I.S.I Hand book of Food Analysis* (part IV). pp 114.

James, C and Sloan, S. 1984. Functional properties of edible rice bran in model systems. *Journal of Food Science*. 29: 310-311.

Javed, M.M., Zahoor, S., Shafaat, S., Mehmooda, I., Gul, A., Rasheed, H., Bukhari, S.A.I., Aftab, M.N and Ikram-ul-Haq. 2012. Wheat bran as a brown gold: nutritious value and its biotechnological applications. *African Journal of Microbiology Research.* 6: 724–733.

Jenkins, D.J.A., Kendall, C.W.C., Augustin, L.S.A., Martini, M.C., Axelsen, M., Faulkner, D., Vidgen, E., Parker, T., Lau, H., Connelly, P.W., Teitel, J., Singer, W., Vandenbroucke, A.C., Leiter, L.A and Josse, R.G. 2002. Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in Type 2 Diabetes. *Diabetes Care*. 25 (9): 1522–1528.

Jisha, S., Padmaja, G and Sajeev, M.S. 2010. Nutritional and textural studies on dietary fiber-enriched muffins and biscuits from cassava-based composite flours. *Journal of Food Quality*. 33: 79–99. Doi: 10.1111/j.1745-4557.2010.00313.x

Johansson-Persson, A., Ulmius, M., Cloetens, L., Karhu, T., Herzig, K-H and Onning, G. 2014. A high intake of dietary fiber influences C-reactive protein and fibrinogen, but not glucose and lipid metabolism, in mildly hypercholesterolemic subjects. *European Journal of Nutrition.* 53 (1): 39–48. Doi: 10.1007/s00394-013-0496-8.

Joseph, N., Nelliyanil, M., Rai, S., Babu, Y.P.R., Kotian, S.M., Ghosh, T., Singh, M. 2015. Fast food consumption pattern and its association with overweight among high school boys in Mangalore city. *Journal of Clinical and Diagnostic Research*. 9 (5): LC13-LC17.

Kadiri, O. 2017. A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing. *International Journal of Food Properties*. 20 (1): S798–S809.

Kalpanadevi, C., Singh, V and Subramanian, R. 2018. Influence of milling on the nutritional composition of bran from different rice varieties. *Journal of Food Science and Technology*. 55: 2259–2269. Doi: https://doi.org/10.1007/s13197-018-3143-9

Kamaljit, K., Amarjeet, K and Pal, S. T. 2011. Analysis of ingredients, functionality, formulation, optimisation and shelf life evaluation of high fiber bread. *American Journal of Food Technology*. 6 (4): 306-313.

Kamble, R. M and Shinde, U.V. 2004. Utility of bran products in Non-Insulin Dependent Diabetes Mellitus (NIDDM) patients. *Journal of Human Ecology.* 16 (3): 219–222.

Karuppasamy. P., Malathi. D., Banumathi, P., Varadharaju, N and Seetharaman, K. 2013. Evaluation of quality characteristics of bread from kodo, little and foxtail millets. *International Journal of Food and Nutritional Sciences*. 2 (2): 46–50.

Kaur, B.J., Gupta, A., Bobade, H., Singh, B and Sharma, S. 2017. Rheological profile and quality assessment of cereal Brans enriched buns, pizza base and flatbread. *International Journal of Chemical Studies.* 5 (6): 1144–1152.

Kearney, J. 2010. Food consumption trends and drivers. *Philosophical Transactions of the Royal Society B*. 365: 2793–2807. Doi: 10.1098/rstb.2010.0149

Kehrer, J.P., Robertson, J.D and Smith, C.V. 2010. Free radicals and reactive oxygen species. *Comprehensive Toxicology.* 277–307. Doi:10.1016/b978-0-08-046884-6.00114-7.

Khawaja, K.I., Fatima, A., Mian, S.A., Mumtaz, U., Moazzum, A., Ghias, M and Masud, F. 2012. Glycaemic, insulin and ghrelin responses to traditional South Asian flatbreads in diabetic and healthy subjects. *British Journal of Nutrition*. 108: 1810–1817.

Kim, B-K., Cho, A-R., Chun, Y-G and Park, D-G. 2013a. Effect of microparticulated wheat bran on the physical properties of bread. *International Journal of Food Sciences and Nutrition*. 64 (1): 122–129.

Kim, M.J., Yoon, W-J and Kim, S.S. 2016. Phytochemical compositions of immature wheat bran, and its antioxidant capacity, cell growth inhibition, and apoptosis induction through tumor suppressor gene. *Molecules.* 21: 1292. Doi: 10.3390/molecules21101292.

Kim, M-J., Park, J.W., Kim, J.Y., Park, K.W., Lee, S-J., Jang, J-K and Lee, J.H. 2013b. Effects of heat treatment and visible light exposure on the oxidative stability of rice bran and of rice bran oil. *Food Science and Biotechnology.* 22 (5): 1223–1228. Doi: 10.1007/s10068-013-0205-7.

Kim, S.M., Rico, C.W., Lee, S.C and Kang, M.Y. 2010. Modulatory effect of rice bran and phytic acid on glucose metabolism in high fat-fed C57BL/6N Mice. *Journal of Clinical Biochemistry and Nutrition*. 47 (1): 12–17.

King, J.C and Keen, C.L. 2003. Zinc. In M. E. Shils, J. A. Olson, M. Shike, C. A. Ross (eds-) *Modern Nutrition in Health and Disease*. 9th ed. NewYork; Lippinkott Williams and Wilkins. pp.223-239.

Kohajdova, Z., Karovičova, J and Jurasova, M. 2012. Influence of carrot pomace powder on the rheological characteristics of wheat flour dough and on wheat rolls quality. *Acta Scientiarum Polonorum, Technologia Alimentaria.* 11 (4): 381-387.

Koh-Banerjee, P., Franz, M., Sampson, L., Liu, S., Jacobs, D.R., Spiegelman, D., Willett, W and Rimm, E. 2004. Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. *American Journal of Clinical Nutrition.* 80: 1237–1245.

Kong, Y.W., Baqar, S., Jerums, G and Ekinci, E.I. 2016. Sodium and Its Role in Cardiovascular Disease - The Debate Continues. *Frontiers in Endocrinology*. 7: 164. Doi: https://doi.org/10.3389/fendo.2016.00164

Krawęcka, A., Sobota, A and Sykut-Domańska, E. 2019. Functional Cereal Products in the Diet for Type 2 Diabetes Patients. *International Journal of Food Science.* 1–7. Doi:10.1155/2019/4012450.

Krings, U., El-Saharty, Y.S., El-Seany, B.A., Pabel, B and Berger, K. 2000. Antioxidant activity of extracts from roasted wheat germs. *Food Chemistry.* 71: 91–95.

Kulamarva, A.G., Sosle, V.R and Raghavan, G.S.V. 2009. Nutritional and Rheological Properties of Sorghum. *International Journal of Food Properties.* 12 (1): 55-69. Doi: 10.1080/10942910802252148

Kumari, B., Randi, K and Sharma, R. 2013. Classification of 3- red sorghum bran and deoxyanthocyanins and its biological properties. *Frontiers of Agriculture and Food Technology*. 1 (7): 063 – 074.

Kumari, D., Madhujith, T and Chandrasekar, A. 2017a. Comparison of phenolic content and antioxidant activities of millet varieties grown in different locations in Sri Lanka. *Food Science and Nutrition.* 5: 474 – 485.

Kumari, R., Bharti, R. K., Singh, K., Sinha, A., Kumar, S., Saran, S and Kumar, U. 2017b. Prevalence of iron deficiency and iron deficiency anaemia in adolescent girls in a tertiary care hospital. *Journal of Clinical and Diagnostic Research*. 11 (8): BC04-BC06.

Kundgol, N.G., Kasturiba, B., Math, K.K., Kamatar, M.Y and Usha, M. 2013. Impact of decortication on chemical composition, antioxidant content and antioxidant activity of little millet landraces. *International Journal of Engineering Research and Technology.* 2 (10): 1705 – 1720.

Küster, I and Vila, N. 2017. Healthy lifestyle and eating perceptions: correlations with weight and low-fat and low-sugar food consumption in adolescence. *Frontiers in Life Science*. 10 (1): 48–62. Doi: 10.1080/21553769.2017.1329170

Kwun, I-S and Kwon, C-S. 2000. Dietary molar ratios of phytate: Zinc and millimolar ratios of phytate × calcium: Zinc in South Koreans. *Biological Trace Element Research.* 75: 29. Doi: https://doi.org/10.1385/BTER:75:1-3:29

Lakkakula, N.R., Lima, M and Walker, T. 2004. Rice bran stabilization and rice bran oil extraction using ohmic heating. *Bioresource Technology*. 92: 157–161.

Lan-Pidhainy, X., Brummer, Y., Tosh, S.M., Wolever, M.T and Wood, P.J. 2007. Reducing beta-glucan solubility in oat bran muffins by freeze-thaw treatment attenuates its hypoglycemic effect. *Cereal Chem*istry. 84 (5): 512–517. Doi: 10.1094/CCHEM-84-5-0512.

Lattimer, J.M and Haub, M.D. 2010. Effects of dietary fiber and its components on metabolic health. *Nutrients*. 2 (12): 1266 – 1289.

Lauková, M., Karovičová, J., Minarovičová, L and Kohajdová, Z. 2019. Wheat bran stabilization and its effect on cookies quality. *Potravinarstvo Slovak Journal of Food Sciences.* 13 (2): 109-115. Doi: https://doi.org/10.5219/1021

Laureati, M., Conte, A., Padalino, L., Nobile. M. A. D and Pagliarini, E. 2016. Effect of fiber information on consumer's expectation and liking of wheat bran enriched pasta. *Journal of Sensory Studies*. 31: 348–359

Lebesi, D.M and Tzia, C. 2011. Effect of the addition of different dietary fiber and edible cereal bran sources on the baking and sensory characteristics of cupcakes. *Food Bioprocess Technology*. 4: 710–722.

Lehtinen, O-P. 2012. Modifying wheat bran for food applications - Effect of wet milling and enzymatic treatment. *Bachelor's Thesis*. Metropolia University of Applied Sciences.

Li, L., Wang, Z., Li, L-M., Zheng, X-L., Ma, S and Wang, X-X. 2018. Effects of fermented wheat bran on flour, dough, and steamed bread characteristics. *Journal of Chemistry.* 1597308. 7 pages. Doi: https://doi.org/10.1155/2018/1597308

Li, Y. O and Komarek, A.R. 2017. Dietary fibre basics: Health, nutrition, analysis, and applications. *Food Quality and Safety*. 1: 47–59. Doi:10.1093/fqs/fyx007.

Liang, S., Yang, G and Ma, Y. 2010. Chemical characteristics and fatty acid profile of foxtail millet bran oil. *Journal of the American Oil Chemists' Society*. 87: 63–67.

Liu, G., Xiao, L., Fang, T., Cai, Y., Jia, G., Zhao, H., Wang, J., Chen, X and Wu, C. 2014. Pea fiber and wheat bran fiber show distinct metabolic profiles in rats as investigated by a 1 H NMR-Based metabolomic approach. *PLoS ONE* 9 (12): e115561. Doi:10.1371/ journal.pone.0115561.

Liu, J., Tang, X., Zhang, Y and Zhao, W. 2012. Determination of the volatile composition in brown millet, milled millet and millet bran by gas chromatography/ mass spectrometry. *Molecules.* 17: 2271-2282.

Liu, N., Ma, S., Li, L and Wang, X. 2019. Study on the effect of wheat bran dietary fiber on the rheological properties of dough. *Grain and Oil Science and Technology.* 2: 1–5.

Lohani, U.C., Pandey, J. P and Shahi, N C. 2012. Effect of degree of polishing on milling characteristics and proximate compositions of barnyard millet (*Echinochloa frumentacea*). *Food Bioprocess Technology*. 5:1113–1119. Doi: 10.1007/s11947-011-0518-6.

Lorenz, K and Dilsaver, W. 1980. Proso millet: Milling characteristics, proximate compositions, nutritive value of flours. *Cereal Chemistry*. 57 (1): 16–20.

Lorenz, K and Hwang, Y. S. 1986. Lipids in proso millet *(Panicum miliaceum)* flours and brans. *Cereal Chemistry*. 63 (5): 387-390.

Loypimai, P., Moongngarm, A. and Chottanom, P. 2015. Stabilization and extraction methods of rice bran. *Emirates Journal of Food and Agriculture*. 27 (11): 849-856. Doi: 10.9755/ejfa.2015.09.738.

Lu, H., Zhang, J., Wu, N., Liu, K-b., Xu, D and Li, Q. 2009. Phytoliths analysis for the discrimination of foxtail millet (*Setaria italica*) and common millet (*Panicum miliaceum*). *PLoS ONE*. 4 (2): e4448. Doi: 10.1371/journal.pone.0004448.

Lubran M.M. 1978. The measurement of total serum proteins by the Biuret method. *Annals of clinical and laboratory science*. 8(2): 106–110.

Luh, B.S., Barber, S and de Barber, B.C. 1991. Rice Bran: Chemistry and Technology Rice. In B.S. Luh (eds). *Rice*. 2nd eds. Springer, Boston, MA.732–781. Doi:10.1007/978-1-4899-3754-4_25

Lv, S.-W., He, L.-Y and Sun, L.-H. 2018. Effect of different stabilisation treatments on preparation and functional properties of rice bran proteins. *Czech Journal of Food Science*. 36: 57–65.

Ma, G., Jin, Y., Piao, J., Kok, F., Guusje, B and Jacobsen, E. 2005. Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China.

Journal of Agricultural and Food Chemistry. 53 (26): 10285–10290. Doi:10.1021/jf052051r

Malleshi, N.G and Hadimani, N.A. 1994. Nutritional and technological characteristics of small millets and preparation of value-added products from them. In Riley, K.W., Gupta, S.C., Seetharman, A., and Mushonga, J.N (eds.) *Advances in Small Millets.* New York: International Science Publisher. pp. 271-287.

Marín-García, J. 2014. Oxidative Stress and Cell Death in Cardiovascular Disease. *Post-Genomic Cardiology*. 471 – 498. doi:10.1016/b978-0-12-404599-6.00014-7

Martianez-Tomea, M., Murcia, M.A., Frega, N., Ruggieri, S., Jimeänez, M.A., Roses, F and Parras, P. 2004. Evaluation of antioxidant capacity of cereal brans. *Journal of Agricultural and Food Chemistry.* 52 (15): 4690-4699.

Masur, S.B., Tarachand, K.C and Kulkarni, U.N. 2010. Effect of incorporation of gluten and wheat bran on quality characteristics of bread flour. *Karnataka Journal of Agricultural Science.* 23 (3): 473-475.

ME, N and Ain, A. 2009. Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malaysian Journal of Nutrition.* 15: 213-22.

Mildner-Szkudlarz, S., Bajerska, J., Górnaś, P., Segliņa, D., Pilarska, A and Jesionowski, T. 2016. Physical and bioactive properties of muffins enriched with raspberry and cranberry pomace powder: A promising application of fruit by-products rich in bio compounds. *Plant Foods for Human Nutrition*. 71: 165–173. Doi: 10.1007/s11130-016-0539-4.

Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L.F., Masana, L., Mangas, A., Hernández-Mijares, A., González-Santos, P., Ascaso, J.F., and Pedro-Botet, J. 2009. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vascular health and risk management*. 5: 757–765.

Mishra, V., Yadav, N., Pandey, S and Puranik, V. 2014. Bioactive components and nutritional evaluation of underutilized cereals. *Annals of Phytomedicine*. 3 (2): 46-49.

Mohapatra, D and Bal, S. 2010. Optimization of polishing conditions for long grain Basmati rice in a laboratory abrasive mill. *Food and Bioprocess Technology*. 3: 466-472. Doi:10.1007/s11947-009-0254.

Mosharraf, L., Kadivar, M and Shahedi, M. 2009. Effect of hydrothermaled bran on physicochemical, rheological and microstructural characteristics of Sangak bread. *Journal of Cereal Science.* 49: 398–404.

Murtaza, N., Baboota, R.K., Jagtap, S., Singh, D.P., Khare, P., Sarma, S.M., Podili K., Alagesan, S., Chandra, T.S., Bhutani, K.K., Boparai, R.K., Bishnoi, M and Kondepudi, K.K. 2014. Finger millet bran supplementation alleviates obesity-induced oxidative stress, inflammation and gut microbial derangements in high-fat diet-fed mice. *The British Journal of Nutrition*. 1121447–1121458. Doi: 10.1017/S0007114514002396.

Mustač, N.C., Novotni, D., Habuš, M., Drakula, S., Nanjara, L., Voučko, B., Benković, M and Ćurić, D. 2020. Storage stability, micronisation, and application of nutrient-dense fraction of proso millet bran in gluten-free bread. *Journal of Cereal Science.* 91: 1-7. 102864. Doi: https://doi.org/10.1016/j.jcs.2019.102864.

Mustač, N.C., Voučko, B., Novotni, D., Drakula, S., Gudelj, A., Dujmic, F and Ćurić, D. 2019. Optimization of High Intensity Ultrasound Treatment of Proso Millet Bran to Improve Physical and Nutritional Quality. *Food Technology and Biotechnology*. 57 (2): 183-190. Doi: https://doi.org/10.17113/ftb.57.02.19.6100

Nagi, H.P.S., Kaur, J., Da, B.N and Sharma S. 2012. Effect of storage period and packaging on the shelf life of Cereal bran incorporated biscuits. *American Journal of Food Technology*. 7 (5): 301-310.

Nandini, C.D and Salimath, P.V. 2001. Carbohydrate composition of wheat, wheat bran, sorghum and bajra with good chapati/roti (Indian flat bread) making quality. *Food Chemistry*.73 (2): 197-203. Doi: https://doi.org/10.1016/S0308-8146(00)00278-8.

Nazni, P and Gomathi, S. 2015. Optimization of fiber rich foxtail millet bran chappatis using response surface methodology. *International Journal of Food and Nutritional Sciences.* 4 (3): 147-151.

Nazni, P and Karuna, T.D. 2016. Development and quality evaluation of barnyard millet bran incorporated rusk and muffin. *Journal of Food and Industrial Microbiology*. 2 (2): 1-6.

Ndlala, F.N., Onipe, O.O., Mokhele, T.M., Anyasi, T.A and Jideani, A.I.O. 2019. Effect of wheat bran incorporation on the physical and sensory properties of a South African cereal fried dough. *Foods.* 8: 559. Doi:10.3390/foods8110559.

Nielsen, O.F., Bilde, M and Frosch, M. 2012. Water activity. *Spectroscopy: An International Journal.* 27 (5-6): 565–569. doi:10.1155/2012/414635

Nilsson, A., Granfeldt, Y., Ostman, E, Preston, T and Bjorck, I. 2006. Effects of GI and content of indigestible carbohydrates of cereal-based evening meals on glucose tolerance at a subsequent standardised breakfast. *European Journal of Clinical Nutrition*. 60: 1092–1099. Doi:10.1038/sj.ejcn.1602423.

Nithya, D.J., Bosco, K.A.S., Mohan, J.R and Alagusundaram, K. 2013. Antioxidant activity of rice bran pasta. *Journal of Microbiology, Biotechnology and Food Sciences.* 2 (6): 2423-2425.

Oghbaei, M and Prakash, J. 2016. Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food and Agriculture* 2: 1136015.

Ogunbusola, E.M., Fagbemi, T.N and Osundahunsi, O.F. 2012. Chemical and functional properties of full fat and defatted white melon (*Cucumeropsis mannii*) seed flours. *Journal of Food Science and Engineering*. 2: 691–696.

Ohkawa, Y., Ohishi, N and Yagi, K. 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*. 95: 351-358.

Oluremi, O.I., Solomon, A.O and Saheed, A.A. 2013. Fatty acids, metal composition and physico-chemical parameters of Igbemo Ekiti rice bran oil. *Journal of Environmental Chemistry and Ecotoxicology*. 5 (3): pp. 39-46.

Onipe, O.O., Jideani, A.I.O and Beswa, D. 2015. Composition and functionality of wheat bran and its application in some cereal food products. *International Journal of Food Science and Technology*. 50: 2509–2518.

Ooi, F.K and Ridzuan, H.M.A. 2016. Combined effects of oat bran supplementation and jogging exercise on body composition and blood lipid profiles in young female.

International Journal of Sports Science. 6 (5): 169-175. Doi: 10.5923/j.sports.20160605.01.

Palaniappan, A., Yuvaraj, S. Sundharam, Sonaimuthu, S and Antony, U. 2017. Characterization of xylan from rice bran and finger millet seed coat for functional food applications. *Journal of Cereal Science.* 75: 296-305.

Pang, M., He, S., Wang, L., Cao, X., Cao, L and Jiang, S. 2014. Physicochemical properties, antioxidant activities and protective effect against acute ethanol-induced hepatic injury in mice of foxtail millet (*Setaria italica*) bran oil. *Food and Function.* 5 (8): 1763–1770. Doi:10.1039/c4fo00106k.

Patel, S. 2015. Cereal bran fortified-functional foods for obesity and diabetes management: Triumphs, hurdles and possibilities. *Journal of Functional Foods.* 14: 255–269.

Patil, S.S., Kara, A and Mohapatra, D. 2016. Stabilization of rice bran using microwave: Process optimization and storage studies. *Food and Bioproducts Processing.* 99: 204–211.

Pauline, M., Roger, P., Nina, N.E.S.N., Arielle, T., Eugene, E. E and Robert, N. 2020. Physico-chemical and nutritional characterization of cereals brans enriched breads. *Scientific African*. Pages 1 – 3. Doi: https://doi.org/10.1016/j.sciaf.2019.e00251

Pawar, V.D and Machewad, G.M. 2006. Processing of foxtail millet for improved nutrient availability. *Journal of Food Processing and Preservation.* 30: 269–279.

Penumarthy, S., Penmetsa, G S and Mannem, S. 2013. Assessment of serum levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in periodontitis patients. *Journal of Indian Society of Periodontology*. 17(1): 30–35. https://doi.org/10.4103/0972-124X.107471

Peryam, D.R and Pilgrim, F.J. 1957. Hedonic scale method of measuring food preferences. *Food Technology*. 11(9): 1-13.

Pinnell, A. E and Northam, B.E. 1978. New automated dye-binding method for serum albumin determination with bromocresol purple. *Clinical chemistry*. 24(1): 80–86.

Poquette, N.M., Gu, X and Lee, S-O. 2014. Grain sorghum muffin reduces glucose and insulin responses in men. *Food and Function*. 5: 894-899.

Prasad, M.P., Rao, B.D., Kalpana, K., Rao, M.V and Patil, J.V. 2015. Glycaemic index and glycaemic load of sorghum products. *Journal of Science and Food Agriculture*. 95 (8): 1626 – 1630. Doi: https://doi.org/10.1002/jsfa.6861

Prashanth, M.R.S., Shruthi, R.R and Muralikrishna, G. 2015. Immunomodulatory activity of purified arabinoxylans from finger millet (*Eleusine coracana, v. Indaf 15*) bran. *Journal of Food Science and Technology*. 52 (9): 6049–6054. Doi: 10.1007/s13197-014-1664-4.

Price, R.K., Welch, R.W., Lee-Manion, A.M., Bradbury, I and Strain, J.J. 2008. Total phenolics and antioxidants potential in plasma and urine of humans after consumption of wheat bran. *Cereal Chemistry.* 85: 152-157.

Purić, M., Rabrenović, B., Rac, V., Pezo, L., Tomašević, I and Demin, M. 2020. Application of defatted apple seed cakes as a by-product for the enrichment of wheat bread. *LWT – Food Science and Technology.* Doi: https://doi.org/10.1016/j.lwt.2020.109391.

Qureshia, A.A., Sami, S.A and Khan, F.A. 2001. Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus Types I and II. *Journal of Nutritional Biochemistry.* 13: 175–187.

Rafe, A., Sadeghian, A and Hoseini-Yazdi, S.Z. 2017. Physicochemical, functional, and nutritional characteristics of stabilized rice bran form Tarom cultivar. *Food Science and Nutrition.* 5: 407 – 414. Doi: https://doi.org/10.1002/fsn3.407

Rahman, R., Hiregoudar, S., Veeranagouda, M., Ramachandra, C.T., Kammar, M., Nidoni, U and Roopa, R.S. 2015. Physico-chemical, textural and sensory properties of muffins fortified with wheat grass powder. *Karnataka Journal of Agricultural Science.* 28 (1): 79-82.

Rajasekaran, A and Kalaivani, M. 2013. Designer foods and their benefits: A review. *Journal of Food Science and Technology.* 50 (1):1–16.

Rajput, S.G., Plyler-harveson, T and Santra, D.K. 2014. Development and characterization of SSR markers in proso millet based on switchgrass genomics. *American Journal of Plant Science*. 5: 175–186.

Ramezanzadeh, F.M., Rao, R.M., Prinyawiwatkul, W., Marshall, W.E and Windhauser, M. 2000. Effects of microwave heat, packaging, and storage temperature on fatty acid and proximate compositions in rice bran. *Journal of Agricultural and Food Chemistry*. 48 (2): 464-467.

Rani, S., Singh, R., Sehrawat, R., Kaur, B. P and Upadhyay, A. 2018. Pearl millet processing: a review. *Nutrition and Food Science*. 48 (1): 30 – 44. Doi: 10.1108/NFS-04-2017-0070.

Rao, H.P and Manohar, R.S. 2003. Chapatis and Related Products. In Caballero, B. (eds.) - *Encyclopedia of Food Science and Nutrition*. 2nd edn. Elsevier Science Ltd. 1033-1044.

Revankar, J., Divya K., Shamnani, A and Podili, K. 2018. Antioxidant activities of Pearl millet (*Pennisetum glaucum*) and Little millet (*Panicum sumatrense*) in different *in vitro* models. *International Journal of Bioassays* 7 (2): pp. 5595-5601.

Rhodes, D. 2014. Diversity, Genetics, and Health Benefits of Sorghum Grain. (Doctoral dissertation). Retrieved from https://scholarcommons.sc.edu/etd/3005

Romjaun, Z.Z and Prakash, J. 2013. Development and assessment of fibre-enriched muffins. *Advances in Food Sciences*. 35 (4): 159-165.

Rupasinghe, H.P.V., Wang, L., Pitts, N.L and Astatkie, T. 2009. Baking and sensory characteristics of muffins incorporated with apple skin powder. *Journal of Food Quality*. 32: 685–694. Doi: 10.1111/j.1745-4557.2009.00275.x

Saccotelli, M.A., Conte, A., Burrafato, K.R., Calligaris, S., Manzocco, L and Del Nobile, M.A. 2017. Optimization of durum wheat bread enriched with bran. *Food Science and Nutrition.* 5:689–695. Doi: https://doi.org/10.1002/fsn3.448

Sadashivam, S and Manickam, A. 2007. *Biochemical Methods*. 3rd ed, New Age International Publishers. Coimbatore. 203-204.

Safdar, R. 2019. To study the effect of high intake of bakery products in causing obesity among students. *EC Nutrition* 14 (10): 829-851.

Sahrir, N.A., Ooi, F.K., Chen, C.K., Kyi, W.M and Meorosman, J. 2017. Effects of oat bran and jogging on aerobic capacity, lipid profile and antioxidant parameters in young sedentary males. *Journal of Physical Education and Sport*. 17 (1): 48-59.

Sairam, S., Krishna, A.G.G and Urooj, A. 2011. Physico-chemical characteristics of defatted rice bran and its utilization in a bakery product. *Journal of Food Science and Technology*. 48 (4): 478–483. Doi: 10.1007/s13197-011-0262-y.

Saleh, A.S.M., Zhang, Q., Chen, J and Shen, Q. 2013. Millet grains: Nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety*. 12: 281-295.

Sanz-Penella, J.M., Laparra, J.M and Haros, M. 2014. Impact of α-Amylase During Breadmaking on In Vitro Kinetics of Starch Hydrolysis and Glycaemic Index of Enriched Bread with Bran. *Plant Foods for Human Nutrition*. 69: 216–221. Doi: 10.1007/s11130-014-0436-7

Saranraj, P and Geetha, M. 2012. Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical and Biological Archives*. 3 (1): 38-48.

Sarma, S.M., Khare, P., Jagtap, S., Singh, D.P., Baboota, R.K., Podili, K., Boparai, R.K., Kaur, J., Bhutani, K.K., Bishnoi, M and Kondepudi, K.K. 2017. Kodo millet whole grain and bran supplementation prevents high-fat diet induced derangements in lipid profile, inflammatory status and gut bacteria in mice. *Food and Function.* Doi: 10.1039/C6FO01467D.

Schlemmer, U., Frolich, W., Prieto, R.M and Grases, F. 2009. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition and Food Research*. 53: S330–S375.

Serna-Saldivar, S.O and Espinosa-Ramırez, J. 2019. Grain Structure and Grain Chemical Composition. In Taylor, J.R.N and Duodu, K.G. *Sorghum and Millets*, 2nd edn. Elsevier Inc with AACCI. 85-129. Doi: https://doi.org/10.1016/B978-0-12-811527-5.00001-0.

Shah, N., Atallah, M.T., Mahoney, R.R and Pellett, P.L. 1982. Effect of dietary fiber components on faecal nitrogen excretion and protein utilization in growing rats. *The Journal of Nutrition*. 112 (4): 658–666. Doi:10.1093/jn/112.4.658

Shan, S., Li, Z., Guo, S., Li, Z., Shi, T and Shi, J. 2014b. A millet bran-derived peroxidase inhibits cell migration by antagonizing STAT3-mediated epithelial-mesenchymal transition in human colon cancer. *Journal of Functional Foods.* 10: 444–455. Doi: https://doi.org/10.1016/j.jff.2014.07.005.

Shan, S., Li, Z., Newton, I.P., Zhao, C., Li, Z and Guo, M. 2014a. A novel protein extracted from foxtail millet bran displays anti-carcinogenic effects in human colon cancer cells. *Toxicology Letters.* 227 (2): 129-138.

Shankar, A.H. 2013. Mineral Deficiencies. In A.J. Magill, D.R. Hill, T. Solomon and E.T Ryan. *Hunter's Tropical Medicine and Emerging Infectious Disease* 9th ed. Elsevier W.B. Saunders. 1003–1010. Doi:10.1016/b978-1-4160-4390-4.00140-5.

Sharif, M.K., Butt, M.S., Anjum, F.M and Nawaz, H. 2009. Preparation of fiber and mineral enriched defatted rice bran supplemented cookies. *Pakistan Journal of Nutrition*. 8 (5): 571-577.

Sharma, H.R., Chauhan, G.S and Agrawal, K. 2004. Physico-Chemical characteristics of rice bran processed by dry heating and extrusion cooking. *International Journal of Food Properties*. 7 (3): 603–614.

Sharma, N and Niranjan, K. 2018. Foxtail millet: Properties, processing, health benefits, and uses. *Food Reviews International*. 34 (4): 329–363. Doi: https://doi.org/10.1080/87559129.2017.1290103.

Sharma, R., Srivastava, T and Saxena, D.C. 2018. Physico-chemical and functional properties of deoiled rice bran and its utilization in the development of extruded product. *The Pharma Innovation Journal*. 7 (5): 109-112.

Sharma, S., Kaur, S., Dar, B.N and Singh, B. 2014. Storage stability and quality assessment of processed cereal brans. *Journal of Food Science and Technol*ogy. 51 (3): 583–588.

Shenoy, A.H and Prakash, J. 2002. Wheat Bran (*Triticum aestivum*): Composition, functionality and incorporation in unleavened bread. *Journal of Food Quality.* 25: 197-211.

Shi, J., Shan, S., Li, H., Song, G and Li, Z. 2017. Anti-inflammatory effects of millet bran derived-bound polyphenols in LPS-induced HT-29 cell via ROS/miR-149/Akt/ NF-κB signaling pathway. *Oncotarget.* 8 (43): pp 74582-74594.

Shi, J., Shan, S., Li, Z., Li, H., Li, X and Li, Z. 2015. Bound polyphenol from foxtail millet bran induces apoptosis in HCT-116 cell through ROS generation. *Journal of Functional Food*. 17: 958 – 968.

Shim, Y.S., Baek, J.W., Kang, M.J., Oh, Y.J., Yang, S and Hwang, I.T. 2016. Reference values for the triglyceride to high-density lipoprotein cholesterol ratio and non-high-density lipoprotein cholesterol in Korean children and adolescents: The Korean National Health and Nutrition Examination Surveys 2007-2013. *Journal of Atherosclerosis and Thrombosis*. 23 (12): 1334 – 1344. Doi: https://doi.org/10.5551/jat.35634

Sieri, S., Brighenti, F., Agnoli, C., Grioni, S., Masala, G., Bendinelli, B., Sacerdote, C., Ricceri, F., Tumino, R., Giurdanella, M.C., Pala, V., Berrino, F., Mattiello, A., Chiodini, P., Panico, S and Krogh, V. 2013. Dietary glycemic load and glycemic index and risk of cerebrovascular disease in the EPICOR Cohort. *PLoS ONE*. 8 (5): e62625. Doi: 10.1371/journal.pone.0062625

Sikwese, F.E and Duodu, K.G. 2007. Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. *Food Chemistry*. 104: 324-331.

Singh, R.P., Murthy, C.K.N and Jayaprakasha, G.K. 2002. Studies on the antioxidant activity of pomogranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *Journal of Agriculture and Food Chemistry*. *50*: 81–86.

Slinkard, K and Singleton, V.L. 1997. Total phenol analysis: Automation and Comparison with manual Methods. *American Journal of Enology and Viticulture*. 28: 49-55.

Smith, J.P., Daifas, D.P., El-Khoury, W., Koukoutsis, J and El-Khoury, A. 2004. Shelf Life and Safety Concerns of Bakery Products—A Review. *Critical Reviews in Food Science and Nutrition*. 44 (1): 19-55. Doi: 10.1080/10408690490263774

Soong, Y.Y., Quek, R.Y.C and Henry, C.J. 2015. Glycemic potency of muffins made with wheat, rice, corn, oat and barley flours: a comparative study between in vivo and in vitro. *European Journal of Nutrition.* 54: 1281–1285. Doi: https://doi.org/10.1007/s00394-014-0806-9

Sreerama, Y.N., Sasikala, V.B and Pratape, V.M. 2009. Effect of enzyme pre dehulling treatments on dehulling and cooking properties of legumes. *Journal of Food Engineering*. 92: 389–395.

Sridevi. 2007. Evaluation of antioxidant properties of whole grains and processed regional cereals of Karnataka. *Master thesis*. University of Agricultural Sciences, Dharwad.

Sridevi., Nirmala, B.B.Y., Hanchinal, R.R and Basarkar, P.W. 2011. Antioxidant contents of whole grain cereals, millets and their milled fractions. *Journal of Dairying, Foods and Home Sciences.* 30 (3): 191–196.

Steinert, R.E., Raederstorff, D and Wolevers T.M.S. 2016. Effect of consuming oat bran mixed in water before a meal on glycemic responses in healthy humans – A Pilot Study. *Nutrients.* 8: 524. Doi:10.3390/nu8090524.

Sudha, M.L., Ramasarma, P.R and Venkateswara Rao, G. 2011. Wheat bran stabilization and its use in the preparation of high-fiber pasta. *Food Science and Technology International.* 17 (1): 47-53.

Suma, K and Nandini, P.V. 2015. Physical and sensory attributes of fibre enriched cookies. *Food Science Research Journal.* 6 (2): 207–214. Doi: 10.15740/HAS/FSRJ/6.2/207-214.

Suma, P. F and Urooj, A. 2012. Antioxidant activity of extracts from foxtail millet (*Setaria italica*). *Journal of Food Science and Technology.* 49: 500–504.

Suma, P. F and Urooj, A. 2014. Antioxidant activity in two pearl millet (*Pennisetum typhoideum*) cultivars as influenced by processing. *Antioxidants.* 3: 55–66.

Takakori, Z., Zare, M. Iranparvare, M and Mehrabi, Y. 2004. Effect of rice bran on blood glucose and serum lipid parameters in Diabetes II Patients. *The Internet Journal of Nutrition and Wellness*. 2 (1): 1–5.

Talukder, S and Sharma, D.P. 2010. Development of dietary fiber rich chicken meat patties using wheat and oat bran. *Journal of Food Science and Technology*. 47 (2): 224–229. Doi: 10.1007/s13197-010-0027-z

Tanabata, T., Shibaya, T., Hori, K., Ebana, K and Yano, M. 2012. *Smart Grain*: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiology*. 160: 1871–1880.

Tao, J. 1989. Rice Bran Stabilization by Improved Internal and External Heating Methods. LSU Historical Dissertations and Theses. 4812. https://digitalcommons.lsu.edu/gradschool_disstheses/4812.

Tao, J., Rao, R and Liuzzo, J. 1993. Microwave heating for rice bran stabilization. *Journal of Microwave Power and Electromagnetic Energy*. 28: 156–164. Doi: 10.1080/08327823.1993.11688217

Taylor, J. R.N. 2016. Millet Pearl: Overview. In Wrigley, C.W., Corke, H., Seetharaman, K and Faubion, J. (eds.) – *Encyclopedia of Food Grains. 2nd eds. Volume 1: The World of Food Grains*. Elsevier Ltd. 190 – 198. Doi: http://dx.doi.org/10.1016/B978-0-12-394437-5.00011-5.

Taylor, J.R and Duodu, K.G. 2015. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products. *Journal of the Science of Food and Agriculture.* 95 (2): 225–237. Doi:10.1002/jsfa.6713

Taylor, J.R.N and Emmambux, M.N. 2008. Gluten-free foods and beverages from millets. In Arendt, E and Bello, F.D. *Gluten-Free cereal products and beverages.* Elsevier, Academic Press: San Diego. 119-141.

Taylor, J.R.N and Kruger, J. 2016. Millets. In Caballero, B., Finglas, P.M and Toldrá, F. (eds.) – *Encyclopaedia of Food and Health.* Elsevier Ltd. 748–757. Doi: 10.1016/b978-0-12-384947-2.00466-9

Taylor, J.R.N. 2019. Sorghum and millets: Taxonomy, history, distribution, and production. In Taylor, J.R.N and Duodu, K.G. *Sorghum and Millets. 2nd eds*. Elsevier Inc with AACCI. 1-19. Doi: https://doi.org/10.1016/B978-0-12-811527-5.00001-0.

Tong, L.-T., Zhong, K., Liu, L., Qiu, J., Guo, L., Zhou, X., Cao, L and Zhou, S. 2014. Effects of dietary wheat bran arabinoxylans on cholesterol metabolism of hypercholesterolemic hamsters. *Carbohydrate Polymers*. 112: 1–5. Doi: 10.1016/j.carbpol.2014.05.061

Tosh, S.M., Brummer, Y., Wolever, T.M.S and Wood, P.J. 2008. Glycemic Response to oat bran muffins treated to vary molecular weight of β-Glucan. *Cereal Chemistry.* 85 (2): 211–217.

Tran, G., 2015. Millet hulls. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/15695. Last updated on May 11, 2015, 14:35.

Tudoricaÿ, C.M., Kuri, V and Brennan, C.S. 2002. Nutritional and physicochemical characteristics of dietary fiber enriched pasta. *Journal of Agricultural and Food Chemistry*. 50 (2): 347-356.

Ugare, R., Chimmad, B., Naik, R., Bharati, P and Itagi, S. 2014. Glycemic index and significance of barnyard millet *(Echinochloa frumentacae)* in type II diabetics. *Journal of Food Science and Technology.* 51 (2): 392–395.

Upadhyaya, H.D., Vetriventhan, M., Dwivedi, S.L., Pattanashetti, S.K and Singh, S.K. 2016. Proso, barnyard, little, and kodo millets. In Singh, M and Upadhyaya, H.D. *Genetic and Genomic Resources for Grain Cereals Improvement*. Academic Press. 321–343.

Ursini, F and Maiorino, M. 2013. *Glutathione Peroxidases. Encyclopedia of Biological Chemistry*. 399 – 404. Doi: 10.1016/b978-0-12-378630-2.00383-2

Veenashri, B.R and Muralikrishna, G. 2011. In vitro anti-oxidant activity of xylo-oligosaccharides derived from cereal and millet brans – A comparative study. *Food Chemistry*. 126: 1475–1481.

Venn, B.J and Green, T.J. 2007. Glycemic index and glycemic load: measurement issues and their effect on diet–disease relationships. *European Journal of Clinical Nutrition.* 61 (1): S122–S131.

Verma, S., Srivastava, S and Tiwari, N. 2015. Comparative study on nutritional and sensory quality of barnyard and foxtail millet food products with traditional rice products. *Journal of Food Science and Technology.* 52 (8): 5147–5155.

Virani S.S. 2011. Non-HDL cholesterol as a metric of good quality of care: opportunities and challenges. *Texas Heart Institute Journal.* 38 (2): 160–162.

Vitaglione, P. Napolitano, A and Fogliano, V. 2008. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*. 19: 451 – 463.

Wang, T., Raddatz, J and Chen, G. 2013. Effects of microfluidization on antioxidant properties of wheat bran. *Journal of Cereal Science*. 58: 380-386.

Wheeler, E.C and Ferrel, P.E. 1971. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*. 48: 312-320.

WHO Expert Consultation. 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 363 (9403): 157 – 63. Doi: 10.1016/S0140-6736(03)15268-3.

Willis, H.J., Thomas, W., Eldridge, A.L. Harkness, L., Green, H and Slavin, J.L. 2011. Glucose and insulin do not decrease in a dose-dependent manner after increasing doses of mixed fibers that are consumed in muffins for breakfast. *Nutrition Research*. 31: 42–47.

Wolevers T.M.S., Jenkins A.L., Prudence, K., Johnson, J., Duss, R., Chu, Y and Steinert, R.E. 2018. Effect of adding oat bran to instant oatmeal on glycaemic response in humans - A study to establish the minimum effective dose of oat β-glucan. *Food Function*. 9 (3): 1692-1700. Doi: 10.1039/c7fo01768e.

Wu, L., Huang, Z., Qin, P., Yao, Y., Meng, X., Zou, J., Zhu, K and Ren, G. 2011. Chemical characterization of a procyanidin-rich extract from sorghum bran and its effect on oxidative stress and tumour inhibition *in vivo*. *Journal of Agricultural and Food Chemistry*. 59: 8609–8615.

Wu, T., Meng, Y., Zhang, W., Liu, R and Zhang, M. 2018. Effects of extrusion on physicochemical properties of oat polysaccharides and its improvement in flour dough extensibility and gumminess. *Food Science and Technology Research*. 24 (1): 145-150.

Xu, J., Chen, X., Ge, Z., Liang, H., Yan, L., Guo X., Zhang, Y., Wang, L and Ma, J. 2017. Associations of Usual 24-Hour Sodium and Potassium Intakes with Blood Pressure and Risk of Hypertension among Adults in China's Shandong and Jiangsu Provinces. *Kidney Blood Pressure Research.* 42: 188 – 200. Doi: 10.1159/000475486.

Yadav, N., Chaudhary K., Singh, A and Gupta A. 2013. Evaluation of hypoglycaemic properties of kodo millet-based food products in healthy subjects. *IOSR Journal of Pharmacy.* 3 (2): 14-20.

Yadav, R. B., Yadav, B. S and Chaudhary, D. 2011. Extraction, characterization and utilization of rice bran protein concentrate for biscuit making. *British Food Journal.* 113 (9): 1173-1182.

Yan, X., Ye, R and Chen, Y. 2015. Blasting extrusion processing: The increase of soluble dietary fiber content and extraction of soluble-fiber polysaccharides from wheat bran. *Food Chemistry*. 180: 106–115

Yaseen, T., Rehman, S. U., Ashraf, I., Ali, S and Pasha, I. 2012. Development and Nutritional Evaluation of Date Bran Muffins. *Journal of Nutrition and Food Sciences.* 2: 124.

Younas, A., Bhatti, M.S., Ahmed, A and Randhawa, M.A. 2011. Effect of Rice Bran Supplementation on Cookie Baking Quality. *Pakistan Journal of Agricultural Science.* 48 (2): 129-134.

Yu, L., Nanguet, A-L and Beta, T. 2013. Comparison of Antioxidant Properties of Refined and Whole Wheat Flour and Bread. *Antioxidants.* 2: 370–383. Doi: 10.3390/antiox2040370

Zeb, A and Ullah, F. 2016. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. *Journal of Analytical Methods in Chemistry*. 5 pages.

Zhang, H.-J., Zhang, H., Wang, L and Guo, X.-N. 2012. Preparation and functional properties of rice bran proteins from heat-stabilized defatted rice bran. *Food Research International*. 47: 359–363.

Zhao, H.-M., Guo, X-N and Zhu, K-X. 2017. Impact of solid-state fermentation on nutritional, physical and flavor properties of wheat bran. *Food Chemistry*. 217: 28–36.

Zhu, Y., Chu, J., Lu, Z., Lv, F., Bie, X., Zhang, C and Zhao, H. 2018. Physicochemical and functional properties of dietary fiber from foxtail millet (*Setaria italic*) bran. *Journal of Cereal Science*. 79: 456–461. DOI: 10.1016/j.jcs.2017.12.011

APPENDICES

Appendix A

Score card for Sensory Evaluation

Name: **Date:**

Product name: **Time:**

Instructions:

- Please evaluate each of the following samples using scoring system given below.
- Write the preferred number score in the column as per evaluation.
- Rinse your mouth in between evaluating each sample.

Code	Appearance	Colour	Texture	Aroma/ Flavour	Taste	Overall Acceptability

Scoring system:

9-Like extremely 8-Like very much 7-Like moderately

6-Like slightly 5-Neithe like nor dislike 4-Dislike slightly

3-Dislike moderately 2-Dislike very much 1-Dislike extremely

Remarks/ Comments:

Signature

Appendix B

PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY, RAJENDRANAGAR, HYDERABAD – 500 030

Informed Consent From (ICF) for students from PG section PJTSAU, who we are inviting to participate in research titled "Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential"

Note: You will be given a copy of the full Informed Consent Form

Part I: Information Sheet

Introduction

I am Barbhai Mrunal D. working as PhD scholar in Foods and Nutrition Department, PGRC, PJTSAU under guidance of Dr. T.V. Hymavathi (Chairman). I am conducting a research on improving nutritional value of bakery products (buns and muffins) as their consumption in all age groups especially adolescents have increased drastically. I invite you to be part of my research, before you decide to participate you may talk about the research to anyone you feel comfortable and take time to decide regarding your participation. The consent form may contain words which you do not understand, so you may ask me your queries and I will explain clearly. Also, if there are any other queries later, you can contact me anytime during the study.

Purpose of research

Consumption of bakery products has drastically increased in recent past in all age groups especially adolescents. These products generally lack nutrients as they are prepared mostly using refined flours, sugars and fat. Changes in lifestyle more towards sedentary one and consumption of foods lacking nutrients tend to associate with health hazards like, obesity, diabetes, cardiovascular disorder and other lifestyle associated diseases. Through our research we want to find ways to make such bakery items (muffins and buns) nutrient rich. The muffins will be developed using combinations of following ingredients: refined wheat flour, kodo millet bran, eggs, oil, milk, sugar, essence, baking powder and salt. Buns will be formulated using refined wheat flour, foxtail millet bran, fat, salt, sugar and yeast. We believe that with your participation we would be able to gather more information regarding the food practices followed by adolescents in general and the glycemic index and glycemic load study would shed some light on the same.

Type of Research Intervention

This research will involve your participation in conducting glycemic index study, where reference food (glucose) providing 25g of available carbohydrate will be provided and after a washout period of 6 days bun/muffin incorporated with millet bran (foxtail / kodo) with same amount of available carbohydrate (as reference food) will be supplemented. Blood samples will be drawn with help of trained medical professional at fasting, 15 min, 30 min, 60, 90 and 120 min after feeding for each products/reference food.

Participant Selection and Voluntary Participation

You are being invited to be a part of this research because you fall into normal healthy group and can contribute aptly to our knowledge and understanding. Your participation in this study is entirely voluntary and you may withdraw anytime between the study.

Procedures and Duration of study

This is a glycemic index study where you will be assessed for oral glucose tolerance test. After washout period of a four days you will be given one serving of reference food (glucose) providing 25 g of available carbohydrate and blood samples will be taken by finger prick test method using glucometer (AccuSure simple, Model No. TD:4183, Manufactured by Taidoc Technology Corporation, Taiwan) at fasting, 15 min, 30 min, 60, 90 and 120 min after feeding. Similar procedure will be followed after washout period of four days in between each product for one serving portion of muffin and bun providing 25 g of available carbohydrate. The fasting blood glucose will be assessed after minimum 10 hours of fasting. There are no risks associated with the consumption of products. In case of any problem faced contact us immediately. Proper measures will be taken to provide assistance. The product is tested for its nutrient content, safety and accordingly the portion sizes are decided. There may not be any direct benefits, but you will definitely contribute to our knowledge and understanding regarding formulation of such products, which will in turn contribute to society. With the current information through various other researches available we assume the product to have low glycemic index which will be understood by end of the study. This information will be part of a PhD research work and will be published under papers and articles for understanding of community.

Confidentiality

The information shared by you regarding your health status will be kept confidential. Your name and other information will be kept confidential, no one outside the research team will know any information shared. The information collected from this research will be kept private and use of any information will be number and not have anyone's name mentioned. Participants anonymity will be maintained.

Right to Refuse or Withdraw

This to let you know that your contribution is valuable, desired, and voluntary, so you may still withdraw if you wish to.

Person to Contact during study

All the queries, doubts are invited now or later. You may clarify you doubts without hesitation and give your opinions/suggestions to me.

Name: Barbhai Mrunal D. (PhD scholar Foods and Nutrition Department)

Mobile: 7259585057

This proposal has been reviewed and approved by the research advisory committee under chairmanship of Dr. T.V. Hymavathi and PJTSAU ethical committee.

Part II: Certification of Consent

I have read the above information, or it had been read to me before signing this consent form. I was given ample opportunities to ask questions regarding the study and received answers to my satisfaction. I consent to voluntary participation in the study and may withdraw my participation from this study at any time without causing any bad feelings. My participation in the study can be terminated by the researchers with proper explanation.

I hereby volunteer to take part in the study and I have received a copy of this consent form.

______________________	______________________	____________
Name of Participant	**Signature of Participant**	**Date**

______________________	______________________	____________
Name of witness	**Signature of Witness**	**Date**

Statement by the researcher/person taking consent

I have carefully read out the information and nature of the study to potential participants and made sure that participant understand nature, demands, benefits, risks of participating in the study. I confirm that participant was given ample opportunity to ask questions and all questions asked were answered correctly and to best of my ability. I confirm that no individual was persuaded into giving consent and the consent was given voluntarily. A copy of this ICF has been provided to the participant.

______________________	______________________	____________
Name of Researcher	**Signature of researcher**	**Date**

______________________	______________________	____________
Name of chairman	**Signature of chairman**	**Date**

Appendix C

PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY, RAJENDRANAGAR, HYDERABAD – 500 030

Informed Consent From (ICF) for adolescent girls from BSc, PJTSAU, who we are inviting to participate in research titled "Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential"

Note: You will be given a copy of the full Informed Consent Form

Part I: Information Sheet

Introduction

I am Barbhai Mrunal D. working as PhD scholar in Foods and Nutrition Department, PGRC, PJTSAU under guidance of Dr. T.V. Hymavathi (Chairman). I am conducting a research on improving nutritional value of bakery products (buns and muffins) as their consumption in all age groups especially adolescents have increased drastically. I invite you to be part of my research, before you decide to participate you may talk about the research to anyone you feel comfortable and take time to decide regarding your participation. The consent form may contain words which you do not understand, so you may ask me your queries and I will explain clearly. Also, if there are any other queries later, you can contact me anytime during the study.

Purpose of research

Consumption of bakery products has drastically increased in recent past in all age groups especially adolescents. These products generally lack nutrients as they are prepared mostly using refined flours, sugars and fat. Changes in lifestyle more towards sedentary one and consumption of foods lacking nutrients tend to associate with health hazards like, obesity, diabetes, cardiovascular disorder and other lifestyle associated diseases. Through our research we want to find ways to make such bakery items (muffins and buns) nutrient rich. The muffins will be developed using combinations of following ingredients: refined wheat flour, kodo millet bran, eggs, oil, milk, sugar, essence, baking powder and salt. Buns will be formulated using refined wheat flour, foxtail millet bran, fat, salt, sugar and yeast. We believe that with your participation we would be able to gather more information regarding the food practices followed by adolescents in general and the supplementation for 45 days would shed some light on the same.

Type of Research Intervention

This research will involve your participation in feeding studies of 45 days, where bun/ muffin incorporated with millet bran (foxtail/kodo) will be supplemented daily. Blood samples will be drawn with help of trained medical professional pre and post supplementation. There will also be set of questionnaires needed to be fulfilled by the you regarding the meals/ foods consumed daily during the study period.

Participant Selection and Voluntary Participation

You are being invited to be a part of this research because you fall into adolescent age group and can contribute aptly to our knowledge and understanding. Your participation in this study is entirely voluntary and you may withdraw anytime between the study.

Procedures and Duration of study

This is a supplementation study where you will be given one serving portion of muffin/bun for 45 day daily to be consumed as part of your breakfast/snack. We require your participation in the study providing information regarding daily meals, food consumed apart from the given supplementation product by filling a questionnaire. We also require to draw blood samples before starting the study and after completion of feeding studies. This will be done with help of trained medical professional in university health center, PJTSAU. Daily visits will be made to clarify problems or doubts. The information shared by you regarding your health status will be kept confidential. There are no risks associated with the consumption of products. In case of any problem faced contact us immediately. Proper measures will be taken to provide assistance. The product is tested for its nutrient content, safety and accordingly the portion sizes are decided. There may not be any direct benefits, but you will definitely contribute to our knowledge and understanding regarding formulation of such products, which will in turn contribute to society. With the current information through various other researches available we assume the product to have therapeutic benefits and antioxidant potentials, which will be understood by end of the study. This information will be part of a PhD research work and will be published under papers and articles for understanding of community.

Confidentiality

The information shared by you regarding your health status will be kept confidential. Your name and other information will be kept confidential, no one outside the research team will know any information shared. The information collected from this research will be kept private and use of any information will be number and not have anyone's name mentioned. Participants anonymity will be maintained.

Right to Refuse or Withdraw

This to let you know that your contribution is valuable, desired, and voluntary, so you may still withdraw if you wish to.

Person to Contact during study

All the queries, doubts are invited now or later. You may clarify you doubts without hesitation and give your opinions/suggestions to me.

Name: Barbhai Mrunal D. (PhD scholar Foods and Nutrition Department)

Mobile: 7259585057

This proposal has been reviewed and approved by the research advisory committee under chairmanship of Dr. T.V. Hymavathi and PJTSAU ethical committee.

Part II: Certification of Consent

I have read the above information, or it had been read to me before signing this consent form. I was given ample opportunities to ask questions regarding the study and received answers to my satisfaction. I consent to voluntary participation in the study and may withdraw my participation from this study at any time without causing any bad feelings. My participation in the study can be terminated by the researchers with proper explanation.

I hereby volunteer to take part in the study and I have received a copy of this consent form.

______________________	______________________	__________
Name of Participant	**Signature of Participant**	**Date**

______________________	______________________	__________
Name of witness	**Signature of Witness**	**Date**

Statement by the researcher/person taking consent

I have carefully read out the information and nature of the study to potential participants and made sure that participant understand nature, demands, benefits, risks of participating in the study. I confirm that participant was given ample opportunity to ask questions and all questions asked were answered correctly and to best of my ability. I confirm that no individual was persuaded into giving consent and the consent was given voluntarily. A copy of this ICF has been provided to the participant.

______________________	______________________	__________
Name of Researcher	**Signature of researcher**	**Date**

______________________	______________________	__________
Name of chairman	**Signature of chairman**	**Date**

Appendix D

Questionnaire (modified using FAO, 2018)

<table>
<tr><th colspan="4">General information</th></tr>
<tr><td colspan="2">Code No:</td><td colspan="2">Date:</td></tr>
<tr><td colspan="4">Name:</td></tr>
<tr><td>Age:</td><td>Gender</td><td>Height</td><td>Weight</td></tr>
<tr><td>BMI:</td><td>Hb</td><td colspan="2">BP:</td></tr>
<tr><td colspan="2">Date of birth:</td><td></td><td></td></tr>
<tr><td colspan="4">Educational qualification:</td></tr>
<tr><td colspan="4">Current address:</td></tr>
<tr><td colspan="4">Permanent address:</td></tr>
</table>

<table>
<tr><th colspan="3">Do you have any of following disease?</th></tr>
<tr><td>Diabetes
Yes
No
If yes, mention your blood glucose level</td><td>Hypertension
Yes
No
If yes, mention your Blood pressure levels</td><td>Thyroid
Yes
No</td></tr>
</table>

<table>
<tr><td>High cholesterol level
Yes
No
If yes, mention your lipid levels</td><td>Polycystic ovary syndrome (PCOD)
Yes
No</td></tr>
<tr><td colspan="2">Are you on any medication?
Yes
No
If yes, mention:</td></tr>
</table>

General food habits			
How many meals you consume in a day?			
1-2 meals	3-4 meals	5-6 meals	6-7 meals
Is there any special meal consumed on weekend		Yes	no
If yes generally what are foods consumed:			
What time do you generally consume meals /snacks?			
Breakfast:	Lunch:	Snacks:	Dinner:
Any additional snack:			
How often do you eat outside?			
What are the foods you eat when you eat outside?			
List the snack items consumed by you frequently			
Any food allergies? Yes NO			

If yes mention								
Frequency of consumption of bakery item								
Item	**Never or rarely**	**1–3 per month**	**1–2 per week**	**3–4 per week**	**5–6 per week**	**Daily**	**2–3 per day**	**5+ per day**
Buns								
Breads								
Muffin/ cupcake								
Cakes								
Biscuits								
Toast								
Burgers								
Pizza								
Any other item missed in above list								
Frequency of consumption of millets								
Jowar								
Bajra								
Ragi								
Foxtail								
Kodo								
Proso								
Barnyard								
Do you exercise regularly?				Yes			No	
Describe your regular exercise routine								
Any other remarks								

24-hour dietary recall

Enter the data regarding all meals consumed

Code No:			**Date:**	
Name:				
Breakfast			**Time**	
Name	**Food description**	**Household Amount**	**Amount (g/mL)**	**Preparation/ Ingredients**
Mid-morning			**Time**	
Lunch			**Time**	

Snacks			**Time**	
Dinner			**Time**	

Additional remarks:

Appendix E

Certificate of Institutional Ethical Committee Approval

PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY

ALL INDIA COORDINATED RESEARCH PROJECT - HOME SCIENCE

POST GRADUATE RESEARCH CENTRE RAJENDRANAGAR, HYDERABAD

Institutional Ethical Committee

Project No. 02 **/IEC/PJTSAU/AICRP-PGRC/** HHD/17-05 **Date :** 13/1/2020

Certificate of Institutional Ethical Committee Approval

This is to certify that, the **Verified Project No.** 02 entitiled Development of Designer Products by Incorporating Millet Bran and assessing their Therapeutic Potential Submitted by Ms. Barbhai Mrunal D. is **APPROVED** by Institutional Ethics Committee, PJTSAU at its meetings held on 20/1/2020, and decision taken by all the IEC members as per the Standard Operating Procedures of the IEC as well as various Ethical Guidelines and Certificate of Ethics Approval to be issued to the Principal Investigator, with advise to the PI that, If any further modification of Post-Ethical Approved Research protocol done by the Principal Investigator, it will be mandatory to submit the modified details WITHIN SEVEN DAYS to the IEC (Institutional Ethics Committee) and if you get clearance regarding modified protocol, then you will be authorized to continue the research work. **The IEC has right to give letter to stop /terminate this study any time.**

Signature
Member Secretary
IEC-PJTSAU
Name: T. KAMALAJA
Date : 20/1/2020

Signature
Chairperson
IEC-PJTSAU
Name: T. V. HYMAVATHI
Date: 20/1/2020

Copy to: * Principal Investigator, *Member Secretary * Office copy

Institutional Ethical Committee Panel for PG/Ph.D Research at AICRP/Department of Foods & Nutrition

Project No. 02/IEC/PJTSAU/AICRP-PGRC/HHD/17-05 Date: 13/11/2020

IEC- Members Signatures			
		Name and Designation	Signature
1.	Chairperson & Convener	Dr. T. V. Hymavathi Professor and University Head, PGRC, PJTSAU, Rajendranagar,Hyderabad-500030	Hymavathi 2d/1/20
2.	Member from AICRP-Home Science	Dr. T. Kamalaja Senior Scientist, AICRP-H.Sc, PGRC, PJTSAU, Rajendranagar, Hyderabad-500030	T. Kamalaja
3.	Member from Department of Foods and Nutrition	Dr. K. Aparna Senior Scientist (F&N), MFPI-QC Lab EEI-Campus, Rajendranagar, Hyderabad-500030	[signature]
4.	Legal expert	Sri. V. Maheswar Assistant Registrar, PJTSAU, Rajendranagar, Hyderabad-500030	[signature]
5.	Clinician	Dr. P. Indrasena Reddy MBBS Diploma Diabetes (NIMS) Medical Officer, PJTSAU Health Centre,Rajendranagar, Hyderabad-500030	P. Indrasena Reddy
6.	Social scientist	Dr. R. Neela Rani Principal Scientist (Extension), AICRP-H.Sc-Extension Component, PJTSAU, Rajendranagar, Hyderabad-500030	N Rani
7.	Psychologist	Dr. P. Sreedevi Assistant Professor, Department of Human Development & Family Studies, College of Community Science, Saifabad, Hyderabad-500004	P. Sreedevi
8.	Nominee from University	Dr. I. Sreenivasa Rao Professor and University Head (Agricultural Extension) EEI, Rajendranagar, Hyderabad-500030	[signature]

The approval of all the members was taken for conduct of Experiment as per the approved research proposal

Signature of Advisor

Signature of Student

Appendix F

Feedback form

1. Rate the products from 1-9

How do you like the product	1st day (Initial)	After 15 days	After 30 days	After 45 days

Scoring system:

9-Like extremely 8-Like very much 7-Like moderately
6-Like slightly 5-Neithe like nor dislike 4-Dislike slightly
3-Dislike moderately 2-Dislike very much 1-Dislike extremely

2. Do you feel there is any change in your approach to select snacks (tick you answer)

Yes

No

If yes what is the change

3. Would you buy if this product is sold in market shelf

Yes:

If yes why

No:

If No why:

Name:

Signature: