Abstract

Minor millets include barnyard millet (*Echinochloa* spp.), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), Guinea millet (*Brachiaria deflexa = Urochloa deflexa*) and brown top millet (*Urochloa ramosa = Brachiaria ramosa = Panicum ramosum*). The major millet type in terms of world production is pearl millet (*Pennisetum glaucum*) which accounts for about 46% of total millet production (Mara thee, 1994) followed by foxtail, proso and finger millets. Millets are important staple food in India and abroad for people of low income groups. Minor millets are arrogated to be future foods for better health and nutrition security. As compared to major cereals such as wheat and rice, millets were found to have high nutritive value. Minor millets are rich in several nutrients as well as non-nutrients such as phenols. Nutritionally, their importance is well recognized because of their high content of calcium (0.38%), dietary fiber (18%) and phenolic compounds (0.3–3%) and is known for several health benefits. Health beneficial effects include anti-diabetic, anti-tumorogenic, atherosclerogenic effects, and antioxidant and antimicrobial properties. The energy of minor millets is greater than other cereals and nearly equal to that of brown rice essentially because their high lipid content (3 to 6%).

Minor millets contain a range of substances which may have health promoting effects. These substances are often referred to as phytochemicals or plant bioactive substances. Millets being a rich source of various phytochemicals including tannins, phenolic acids, flavonoids, anthocyanins, and phytosterols and policosanols have potential of significant influence on human health and well being. A majority of bioactive compounds of whole-millet grains are present in the bran/germ fraction of cereal-grains. Millets have been recommended as nutraceuticals or functional food due to the presence of essential dietary components in
appreciable amounts such as dietary fibers, proteins, minerals, vitamins, and antioxidants necessary for human health.

Germination is one of the most common techniques used for improving the nutraceuticals value of minor millet grains and to reduce most of the anti-nutritional factors. In recent investigations, it has been observed that well-designed soaking and germination techniques can significantly decrease the phytate and tannin contents in food grains similarly germination under controlled and predefined conditions has the ability to enhance the concentration of already present ingredients and also synthesize the new bioactive components in the food grains. The incorporation of germinated grains flour in the product formulation may improve nutritional quality, increase digestibility and bioavailability of some nutrients through reduction of antinutrient.

Hence, the present study was undertaken to characterize the raw and germinated minor millets flour to evaluate the effect of bio-processing on millet essential ingredients and their utilization in suitable food products like cookies, cakes and also to evaluate their quality characteristics and nutritional characteristics. Study also includes extraction of some bioactive compounds from raw and germinated minor millet grains and their application in product development and characterization. The present work has been compiled into nine Chapters.

Chapter one deals with introduction part, in which different aspects of foxail millet, barnyard millet and kodo millet such as nutritional composition, bioactive compounds and malting/germination process has been discussed. Second chapter begins with the literature review of the chemical constituents and bioactive compounds of minor millet grains and effect of malting/germination on minor millet grain constituents, bioactive compounds and potential
health benefits of minor millet followed by product applications. Utilization of minor millet grains flour in different food products and its nutritional evaluation have also been discussed in this chapter.

The Chapter three includes optimization of germination process and effect of the processing parameter of selected raw material such as foxtail, barnyard and kodo millet grain (raw and germinated) to prepare flour. The optimal conditions for germination of foxtail, barnyard and kodo millets were soaking time (13.52 h), germination temperature (25°C ) and germination time (40 h); Soaking time (11.75 h), germination temperature(32.75°C), germination time (36.75 h); and soaking time (12.62 h), germination temperature (34.84°C) and germination time (31.77 h), respectively. Under these optimal conditions, foxtail, barnyard and kodo millet grains flour had high phenolic contents, flavonoid contents and antioxidant activities. The characterization viz. chemical composition, mineral content, dietary fiber and anti-nutritional factors of optimized raw and germinated foxtail, barnyard and kodo millet grains flour have also been discussed in Chapter -3. Germinated foxtail, barnyard and kodo millet flour had shown significantly (p≤0.05) higher protein, crude fibre, dietary fiber and mineral contents while contained significantly lower value for ash, fat, carbohydrate and anti-nutritional factors viz. tannins and phytates than raw foxtail, barnyard and kodo millets flour. Raw foxtail millet contained highest amount of tannins (2.807 mg/100g), followed by raw kodo and barnyard millet (1.603 and 1.594 mg/100 g), respectively. The phytic acid contents, for all the germinated millet samples, were reduced significantly by 0.102 mole/kg, 0.099mole/kg, and 0.997 mole/kg, in germinated foxtail, barnyard and kodo millets, respectively. Germinated foxtail millet possessed highest protein content (14.32%) followed by barnyard millet (11.22%) and kodo millet (7.8%). The total dietary fiber contents in foxtail, barnyard and kodo millet flour increased after
germination bioprocess. The total dietary fiber of raw and germinated kodo were higher than raw and germinated foxtail and barnyard millet because higher amount presence of soluble fiber in kodo millet.

The characteristics of three functional constituents’ viz. phenolic content, $\gamma$-amino butyric acid ($GABA$) and $\beta$-glucan extracted from the raw and optimized germinated foxtail, barnyard and kodo millets flour are elaborated in Chapter-4. Compared to the raw and optimized germinated millet, the free, bound and the total phenolics content increased significantly ($p<0.05$) from 9.79 to 21.75 mg (GAE)/100g, 24.38 to 35.42 mg (GAE)/100g and 34.17 to 35.42 mg(GAE)/100g, respectively in foxtail millet; from 11.46 to 32.22 mg(GAE)/100g, 17.55 to 25.46 mg(GAE)/100g and 29.01 to 77.68 mg(GAE)/100g, respectively in barnyard millet and from 16.46 to 44.45 mg(GAE)/100g, 38.08 to 39.56 mg(GAE)/100g and 54.54 to 84.01 mg(GAE)/100g, respectively in kodo millet. The free, bound and total flavonoid contents were highest in optimized germinated kodo millet followed by that of germinated barnyard millet and germinated foxtail millet. Similarly the free, bound and total flavonoid contents of kodo millet increased after germination from 19.25 to 48.57 mg RU/g, 34.34 to 38.96 mg RU/g and 52.95 to 87.43 mgRU/g, respectively, followed by barnyard millet from 9.48 to 45.57 mg RU/g, 19.54 to 26.35 mg RU/g and 29.02 to 71.92 mgRU/g, respectively. The increase in concentration of some phenolic contents as well as synthesis of some new compounds analyzed by GC-MS was observed in flour after germination.

The *in vitro* antioxidant activities were modified by the germination process, as measured by metal chelating activity, reducing power, total antioxidant capacity, DPPH scavenging and hydrogen peroxide scavenging activity assays as shown in Chapter-4. The total antioxidant activity increased after germination from 29.0 to 45.23 mgAAE/g, 36.45 to 49.12 mgAAE/g and
45.34 to 67.23 mgAAE/g in foxtail, barnyard and kodo millet, respectively. The metal chelating activities of the kodo millet seed extract increased from 62.34 to 89.32 mgEDTA/g, respectively after germination followed by barnyard millet from 48.32 to 76.34 mgEDTA/g and that of foxtail millet from 34.92 to 57.38 mgEDTA/g. The reducing power of germinated millets extract was higher as compared to raw millet extracts and the kodo millet possessed the highest reducing power ability as compared to foxtail and barnyard millets. The DPPH radical scavenging of the raw and germinated kodo millet seed extract from 67.34 to 76.34%, respectively followed by barnyard millet from 47.34 to 68.32% and foxtail millet from 48.32 to 59.62%.

The GABA content also increased from 7.15 to 38.5 mg/100 g, from 6.36 to 35.7 mg/100 g and 9.36 to 47.4 mg/100 g in germinated foxtail, barnyard and kodo millets, respectively, as compared to raw millet samples. The antioxidant capacity of γ-amino butyric acid was determined by three methods such as scavenging ability on DPPH radicals, total antioxidant activity, and hydrogen peroxide scavenging activity. The results indicated that the antioxidant capacity of γ-amino butyric acid was significantly increased (p<0.05) during the germination process as estimated by these three methods. The DPPH radical scavenging activity increased after germination in foxtail, barnyard and kodo millet from 48.32% to 55.62%, from 45.34 to 65.34% and 44.70% to 70.210% and the total antioxidant activity from 12.60 millimole/g to 44.24 millimole/g, 15.3 millimole/g to 34.3 millimole/g and 18.20 millimole/g to 50.24 millimole/g, respectively. While as the hydrogen peroxide scavenging activity in GABA extract of the raw and germinated foxtail, barnyard and kodo millet increased from 35.44 to 64.07 millimole/g, from 38.4 to 65.7 millimole/g, and 40.52 to 68.74 millimole/g, respectively.

The yield of β-glucan gum extract obtained was 4.78% in raw foxtail millet, followed by 4.99% in germinated foxtail millet, 5.12% in raw Kodo millet and 4.66% in germinated kodo
As the extracted pellets did not represent the whole quality of β-glucan isolate, the percent recovery was identified and it was found to be in raw and germinated foxtail and kodo millets extracts increased from 72.17 to 77.44 % and 86.91 to 76.34 %, respectively. The yield of β-glucan gum obtained from raw and germinated barnyard millet flour was 4.85% and 4.27%, respectively, whereas, the percent recovery was found increased from 79.17% and 75.17 %, respectively in raw and germinated barnyard. The results of chemical characteristics, the water binding capacity, swelling power, the foaming capacity and stability, antioxidant activity and the metal chelating ability of β-glucan extract of minor millet flour indicated a significant increase after germination. The water binding capacity and swelling power of β-glucan of raw and germinated kodo millet was found 4.45 to 4.99 g/g and 2.54 to 2.99 g/g and was higher than raw and germinated foxtail having value ranging from 2.88 to 3.06 g/g and 1.32 to 1.67 g/g and barnyard millet 2.13 to 2.32 g/g and 1.45 to 1.76 g/g, respectively. The foaming capacity and stability of β-glucan gum extract of foxtail and barnyard millet increase significantly from 150 g/g to 167 g/g and 59.20 g/g to 65.23 g/g, 156 g/g to 163 g/g and 54.20 g/g to 62.23 g/g, respectively that of Kodo millet increased from 165 g/g to 173 g/g and 58.34 to 64.34 g/g, respectively, after germination. The antioxidant activity of β-glucan extract of kodo millet after the germination was increased significantly from 55.94 to 78.74%, followed by barnyard millet from 44.39 to 57.42 % than foxtail millet from 24.77 to 34.96 %. The metal chelating ability of β-glucan extract of raw and germinated kodo millet was found 44.67 to 48.98 mg EDTA/g extract and was higher than raw and germinated foxtail millet extract (29.56 to 38.67 mg EDTA/g) and for barnyard millet the value ranged from 34.56 to 39.67 mg EDTA/g.

The coefficients of determination (R^2) obtained for the β-glucan extracts was closed to 1, indicating that power law model was adequately suitable for describing the flow behaviour of the
samples. The results showed that ‘n’ value was less than unity confirming that these products behaved like *pseudoplastic* materials. The apparent viscosity of β-glucan extracts for the millets significantly decreased during germination. Germinated foxtail and barnyard millets possessed higher power law exponents, 0.84 due to relatively low β-glucan contents which represented greater shear-thinning capacity as compared to raw millets. Similarly the raw kodo millet which had relatively high β-glucan contents, possessed smaller power law exponents, 0.68 as compared to germinated kodo millet having power law exponent of 0.76 and represented greater shear-thinning capacity as compared to raw kodo millet. The values of the apparent viscosity and consistency index (K) for β–glucan extracts of raw and germinated kodo millet were higher than the raw and germinated β-glucan extracts of foxtail and barnyard millet. Overall, the $G'$ was greater than $G''$ throughout the measured frequency range of 0.01 to 10 rads/s for β-glucan extracts of raw and germinated foxtail, barnyard and kodo millets, indicating the property of these materials could be classified rheologically as elastic gels. In the present study, the $(G'/\gamma)$ and $(G''/\gamma)$ values of β-glucan gum of barnyard millet before germination were 399 Pa and 365 Pa, respectively, and after germination the $(G'/\gamma)$ and $(G''/\gamma)$ were 279 Pa and 245Pa, respectively. β-glucan extracts of raw and germinated foxtail millet indicated peaks values for $G'$ and $G''$ as 203 Pa; 111 Pa, and 178 Pa; 99 Pa, respectively, and that of β-glucan extracts of raw and germinated kodo millet having $G'$ and $G''$ values of 423Pa, 341 Pa and 356Pa; 271Pa respectively. The results of peak tan δ, in the given β-glucan extracts of raw and germinated foxtail millets were found to be ≤ 1 and found 0.5467 and 0.5561, respectively. The results of peak tan δ, in the given β-glucan of raw and germinated barnyard millet were found to be ≤ 1 and ranged from 0.7046 and 0.7840, respectively. However the β-glucan extracts of raw and kodo millet had shown the highest values of peak tanδ, 0.8061 and δ 0.7612, respectively.
β-glucan of raw and germinated foxtail millet behaved as a solid like mass because the magnitudes of K', 37.45 & 29.23 respectively, were much greater than those of K", 28.95 & 20.14, with a high dependency (n' = 0.36–0.51, n" = 0.49–0.78) on frequency, whereas, β-glucan extracts of kodo millet behaved much as viscoelastic as compared to β-glucan extract of foxtail and barnyard millet because the magnitudes of K', 47.52 & 31.87 and those of K", 38.22 & 26.22 respectively, with a high dependence (n' = 0.28–0.46, n" = 0.33–0.54) on frequency. The complex viscosity of β-glucan of raw and germinated foxtail, barnyard and kodo millets decreased as the frequency increased from 0.01 to 10 rad/s, although the lowest complex viscosity was observed at the higher frequencies which indicated that beta glucan of raw and germinated foxtail and kodo millets showed a shear thinning behaviour.

The fifth chapter deals with the preparation and characterization of cookies. Six flour blends (A₁, A₆) were prepared by homogenously mixing raw and germinated foxtail millet, barnyard millet and kodo millet flours as per formulations which were later used to make cookies. Analyses (physicochemical, total phenolic content, in-vitro antioxidant, pasting, textural, nutritional, colour, and sensory characteristics) were carried out for flour blends as well as cookies to evaluate the processing effects. Analyses revealed that germinated flour blends contained highest proteins/nitrogenous material, total phenolics and possessed high in-vitro antioxidant activity, less fat and carbohydrate contents than raw flour blends. A significant difference was observed in the phenolic contents and DPPH radical scavenging activity in flour blends of raw and germinated minor millets. The in vitro antioxidant activity of flour blends of germinated minor millets varied from 58.23 % to 89.23%. The flour blend (A₆) of germinated minor millets had the highest in vitro antioxidant activity (89.23%) whereas, the flour blend (A₁) of raw minor millet having the lowest (55.23 %) in vitro antioxidant activity. Germination had a
negative effect on pasting characteristics whereas functional properties were significantly improved of the flour blends. Water absorption capacity (WAC) of blended flour samples (A1-A6) of raw foxtail, barnyard and kodo millet varied from 1.46 g/g to 2.57 g/g and water absorption capacity of blend flour sample of germinated foxtail, barnyard and kodo millet varied from 2.01 to 4.68 g/g when compared to control (1.39 g/g). For blended flour samples of germinated minor millet flours WSI of raw foxtail, barnyard and kodo millet varied from 2.25 g/g to 7.23 g/g. In comparison to germinated blends of millet flours, the bulk density of the raw blends of millet flour samples were significantly high (p< 0.05). Bulk densities of flour blends of germinated minor millet were lower as compared to flour blends of raw minor millet flour.

Pasting temperature of the raw and germinated minor millet blended flour sample ranged from 78.00°C to 86.85°C and 77.68 to 85.20°C respectively, being the highest for blended flour sample (A2) of germinated minor millet (85.20°C) and lowest for blended flour sample (A6) of germinated minor millet (77.68°C). The ranges observed in trough viscosity of blended flour samples the raw and germinated minor millet were 87 to 305 cP and 35 to 76 cP, respectively. The lower setback value of blended flour samples (A1-A6) of germinated minor millet (16 to 79 cP) as compared to blended flour samples of (A1-A6) of raw minor millet (93 to 326 cP) indicated their lowest rate of retrogradation and hence the product made of low set back viscosity flour will have prolonged shelf life period. Cookies prepared from raw minor millets flour blends showed highest spread ratio, followed by cookies from germinated flour blends and wheat flour. Snap test values were lowest for cookies made from germinated flour blends whereas phenolic content (45.43 mg/100g), DPPH activity (42.34%), dietary fibre (12.36g/100g) and nutritional value were highest followed by cookies prepared from raw minor millets and control. The highest mean score (7.85) for overall sensory attributes were observed for cookies produced by incorporating
germinated foxtail, barnyard and kodo millet in the proportion of 70:20:10 (A₂) than control cookies (7.23 as well as for cookies produced by incorporating raw foxtail, barnyard and kodo millets (6.75) in same proportion of 70:20:10. This indicated that (A₂) sample cookies produced by incorporating germinated foxtail, barnyard and kodo millet in the proportion of 70:20:10 were more acceptable in all respects.

The sixth chapter covers the cake preparation process and its characterization. The study was carried out to investigate the effect of addition of soluble dietary fiber β-glucan gum (2.5 to 7.5%) extracted from minor millets on physicochemical, textural, sensory and in vitro antioxidant characteristics of cakes. The moisture, protein, and ash contents of the cake increased with increase in the β-glucan content from 2.5 to 7.5%. The protein content varied from 9.93 to 10.45% while the moisture content was increased from 16.32 to 17.99%. The ash content and crude fibre slightly increase from 2.40 to 3.53% and form 2.64 to 3.54%, respectively. The total dietary fiber of cake flour varied from 4.34 to 9.56 g/100 g when the level of β-glucan was increased from 2.5 to 7.5%, whereas, the dietary fiber content of control flour was 1.84 g/100 g. The water activity of cake samples containing β-glucan of from all the three sources increased significantly from 0.52 to 0.60 with the addition of β-glucan content from 2.5 to 7.5%. The volume index of cake was improved with β-glucan addition from 2.5 to 5.0% and decreased with further addition of β-glucan up to 7.5%. β-glucan addition significantly improved cohesiveness, springiness, chewiness, of the cake as compared to control sample where as firmness decreased with increase in β-glucan content. Panelists rated highly the cake fortified with β-glucan levels up to 5.0% of germinated millets and were found acceptable in terms of sensory score having similarities to that of control cake. The decrease in sensory score at higher level of β-glucan could be correlated with TPA which had shown increased gumminess, cohesiveness and
decrease in resilience score. The ‘L’ & ‘b’ values of cake crust as well as crumbs decreased significantly with increase in β-glucan levels, whereas, ‘a’ value increased significantly. Overall, addition of up to 5.0% β-glucan gum extract in wheat flour cake significantly improved its characteristics. The in vitro antioxidant activity of cake samples increased from 18.23 to 22.58% when the level of β-glucan was increased from 2.5 to 7.5% in cake, whereas less increase in the antioxidant activity was observed in case of β-glucan of foxtail and barnyard millet as compared to kodo millet. The cake samples fortified with β-glucan (5.0 %) of germinated millets were optimized on the basis of physical, textural and sensory properties. The cake fortified with β-glucan (5.0%) of germinated kodo millet was found acceptable in terms of sensory score. The control cake and cake fortified with 5.0% β-glucan of germinated kodo millet were liked more than the cake fortified with 2.5 and 7.5% β-glucan of germinated kodo millet in terms of crumb/crust color, crumb/crust texture, odor and overall acceptability.

In the seventh Chapter, nutritional evaluation and storage stability (shelf life stability under variable conditions of storage and packaging materials) of cookies and cake have been discussed. Quality characteristics such as moisture, a_w, texture profile, over all acceptability (OAA) and shelf stability parameters viz. free fatty acid (FFA) and peroxide value (PV) of gluten free cookies made from raw and germinated minor millets based composite flour were investigated during accelerated storage at 25±2°C and 50% RH for 120 days packed in two different packaging materials (LDPE & MET-PPE). Results showed that germinated foxtail, barnyard and kodo blended flour cookies represent higher nutritional value than raw millets flour blend cookies. Packaging materials and storage time had significant effect on moisture content and a_w of cookies. Hardness decreased with increase in storage time of cookies packed in both METPPE and LDPE, whereas, decrease was more in cookies packed in LDPE laminate as
compared to cookies packed in METPPE laminated. During storage, moisture content and $a_w$ increased more in LDPE as compared to MET-PPE packed cookies. The FFA and PV of cookies increased in both packaging material during storage whereas, highest increase was observed in cookies packed in LDPE as compared to MET-PPE laminate. The highest average OAA was fetched for cookies packed in MET-PPE laminate. The MET-PPE was found better packaging material than LDPE with respect to sensory, texture and shelf stability characteristics of cookies. The effects of two different temperature i.e. low temperature (refrigerator at 7°C) and ambient temperature (25°C) were observed on moisture content, peroxide value, free fatty acid, firmness, staling, microbial counts and sensory score of control cake and cake fortified with 5.0% $\beta$-glucan of kodo millet during storage stability study. Higher quality changes were observed in control cake and cake fortified with 5% of $\beta$-glucan stored at room temperature than stored at refrigerator temperature. Cake fortified with 5% of $\beta$-glucan showed the lowest peroxide value and the free fatty acid throughout storage period than control sample. This may be due to $\beta$-glucan which binds water acts as antioxidants and prevent the lipid peroxides formation during storage and delayed oxidation. The texture results showed that the firmness values of fortified cake with $\beta$-glucan after eight days of storage were similar or lower than those of the respective controls cake samples. The fortified cake with $\beta$-glucan presented softer crumb than the control cake, implying an anti-staling action. The overall acceptability data showed higher decrease in the sensory scores of control cake and cake fortified with 5% of $\beta$-glucan stored at room temperature than stored at refrigerator temperature. The results from microbiological analysis suggested that low storage temperature and addition of $\beta$-glucan prolongs the microbiological shelf life of cake.
Based on above observations, the results have been summarized and conclusions drawn from these findings have been manifested in eighth chapter. Results suggested that the raw as well as germinated foxtail, barnyard and kodo millet flour have abundant nutritional profile for manufacture of nutrient-rich gluten-free products. Furthermore, results indicated considerable variations in chemical composition and bioactive compounds of the foxtail, barnyard and kodo millet flours after germination. Studies showed that germinated foxtail, barnyard and kodo millet blend flour cookies were of more acceptable quality than raw foxtail, barnyard and kodo millet blend flour cookies, while cake fortified with 5% beta glucan had high dietary fiber and beta glucan contents than control cake. Results of nutritional evaluation showed that cookies represent high nutritional value and can be effectively stored up to 120 days at ambient conditions when packed in standard packaging materials.