Summary and Conclusion

In the present study we have observed how germination/malting affect the nutritional value and nutraceutical properties of minor millet grains. Minor millet is considered as a staple food in most of the countries. Hence there is always a need to improve the nutraceutical properties of minor millet grains. The study also included the extraction process for extraction of bioactive compounds from foxtail, barnyard and kodo millet (raw and germinated) grains, and further characterization of extracted bioactive compounds have been discussed. The foxtail, barnyard and kodo millet (raw and germinated) flour have been used to prepare wholesome cookies. The effect of addition of beta glucan extract of germinated minor millet on cake properties as shown in the present study. The developed products were evaluated for nutritional composition and storage stability.

The germination process parameters for foxtail, barnyard and kodo millet were optimized using CCRD design of response surface methodology. Experiments were carried out to find whether soaking time, germination time and germination temperature had significant effect on nutritional composition, phenolic content, flavonoid content, antioxidant activities, dietary fiber, GABA content and β-glucan content of minor millet grains during germination. The foxtail, barnyard and kodo millet (raw and germinated) flour was analyzed for nutritional, the ant nutritional factor such as tannin and phytic acid present in minor millet which reduced during the course of germination. Raw foxtail millet contained the 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 content, for all the germinated millet samples, was reduced significantly by 0.102mole/kg, 0.099mole/kg, and 0.997mole/kg, in germinated foxtail, barnyard and kodo millet, respectively.
Germinated foxtail millet possessed highest protein content 14.32 % followed by barnyard millet 11.22 % and kodo millet 7.8 %. The total dietary fibre or fiber contents in foxtail, barnyard and kodo millet flour increased after germination bioprocess 27.42 %, 23.74% and 38.4%, respectively. The total dietary fiber in raw and germinated kodo millet higher than raw and germinated foxtail and barnyard millet because higher amount presence of soluble fiber in kodo millet. After the germination, there was a significant increase in mineral content iron (Fe), calcium (ca), magnesium (Mg) and sodium (Na) was observed, amongst the millet samples.

The yield of phenolic content, GABA extract in foxtail, barnyard and kodo millet showed a significant increase during the germination process degradation occurred in β-glucan yield after germination. The free, bound and total phenolic content and the free, bound and total flavonoid was higher in raw and optimized germinated kodo millet as compared to raw and optimized germinated foxtail and barnyard millet. As Comparing to the raw and optimized germinated millet, in foxtail millet 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 barnyard millet free, bound and the total phenolics content increased significantly (p<0.05) from 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 and in kodo millet free, bound and the total phenolics content increased significantly (p<0.05) from 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.

From the results it was observed the metal chelating activity, reducing power, total antioxidant capacity, DPPH scavenging and hydrogen peroxide scavenging activity assays of foxtail, barnyard and kodo millet extract was significantly (p< 0.05) affected by the germination process. The metal chelating activities of the raw and germinated kodo millet seed extract from
62.34 to 89.32 mgEDTA/g, respectively followed by barnyard millet from 48.32 to 76.34 mgEDTA/g and foxtail millet from 34.92 to 57.38 mgEDTA/g. Among the three minor millet the kodo millet possessed highest the metal chelating activity, reducing power, total antioxidant capacity, DPPH scavenging and hydrogen peroxide scavenging activity assays as compared to the other millets due to presence of high amount of phenolic and flavonoid content in kodo millets. The eighteen phenolic compounds were identified and quantified by GC-MS analysis in germinated foxtail, barnyard and kodo millet flour having various health benefits. The results showed that germination increased the concentration of phenolic compounds and new compounds synthesis.

The GC-MS analysis of raw/ foxtail, barnyard and kodo millet revealed the presence of number of new compounds in foxtail millet such as, Astaxanthin (2.14%), Propanoic acid (2.06%), Monolinoleoylglycerol trimethylsilyl ether (2.17%), 9,12,15Octadecatrienoic Acid (2.18%) and Pentadecanoic acid, 14methyl, methyl ester(0.73%) while in the germinated Kodo millet such as, nPropyl 9,12,15octadecatrienoate(0.86%), Pregan20one, 2,hydroxy5, 6epoxy15,methyl (4.45%), Hexadecanoic acid (8.19%), 9Octadecenoicacid (5.00%), Butyl 6,9,12, 15,octadecatetraenoate (4.03%), Hexadecanoic acid, methyl ester (1.43%), compounds indentified as markers but not in the un-germinated samples. In barnyard millet new compounds occurred after germination which act as markers such as Phthalic acid, hept4yl isobutyl ester (25.32 %), Phthalic acid, butyl hex3yl esterPhthalic acid, butyl hex3yl ester (5.96 %), 9,12Octadecadienoic acid, ethyl ester (11.00), Lupeol (5.16 %) and Campesterol (7.59 %). These compounds since were synthesized during the process of germination (sprouting) and therefore can be used as the germination markers.
The yield of GABA also remarkably increased, by 11.25%, 12.34 % and 11.99 %, in germinated foxtail, barnyard and kodo millet respectively, compared with that of raw kodo millet sample whereas the purity of GABA content was ranged in foxtail, barnyard and in Kodo millet from 7.15 to 38.5 mg/100 g, from 6.36 to 35.7mg/100g and 9.36 mg to 47.4g/100g, respectively. The yield of GABA extract low in germinated kodo millet but the purity of GABA content higher than other germinated millet. Antioxidant capacity of γ- amino butyric acid determined by three methods has been shown in present study such as scavenging ability on DPPH radicals, total antioxidant activity, and hydrogen peroxide scavenging activity. 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, 15.3millimole/g to 34.3millimole/g and 18.20 millimole/g to 50.24 millimloe/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 65.7millimole/g, and 40.52 to 68.74 millimole/g, respectively.

The yield of β-glucan extracts of foxtail, barnyard and kodo millet decreased during the germination. Antioxidant capacity of γ- amino butyric acid determined by three methods such as scavenging ability on DPPH radicals, total antioxidant activity, and hydrogen peroxide scavenging activity. The yield of β-glucan extracts (5.65%) and the percent purity of β-glucan was 84.50% in germinated Kodo millet was higher than β-glucan extract (4.99%) of germinated foxtail and barnyard millet. Germination significantly improved the functional properties, chemical composition and antioxidant activity of β-glucan. The apparent viscosity of β-glucan extracts for foxtail, barnyard and kodo the millets significantly decreased during germination.
which may be attributed to reduction in soluble fiber and depolymerisation of macromolecules due to germination as the viscosity depends more on the soluble fiber than insoluble fiber. The values of the apparent viscosity and consistency index (K) for β–glucan of raw and germinated kodo millet greater than β–glucan of foxtail millet and barnyard millet, β-glucan of kodo millet possessed greater hydration capacity and thickening properties. The anti-oxidant activity of β-glucan extract of minor millet significantly increases during germination. The elastic modulus $G'$ was greater than viscous $G''$ throughout the measured frequency (from to 0.01 to 10) range for β–glucan of raw and germinated foxtail, barnyard and kodo millet, indicating that the property of these materials could be classified rheologically as elastic gels. The β-glucan of kodo millet behave as a more viscoelastic properties as compared to β-glucan of foxtail and barnyard millet because the magnitudes of $K'$ (from 31.87 to 47.52), and those of $K''$ (26.22 to 38.22), were much greater than β-glucan of foxtail and barnyard millet with a high dependence ($n' = 0.16–0.28$, $n'' = 0.33–0.49$) on frequency. The storage and loss modulus of β-glucan germination led to a dramatic decrease in both $G'$ and $G''$ for beta glucan gum of foxtail, barnyard and kodo millet. The present research revealed that although germination reduced the yield of β-glucan in foxtail, barnyard and kodo millets but there were a significant increase in the purity of β-glucan in the extract along with improvement in the physicochemical characteristics. The results indicated that after germination of millets, the $G'$ of β-glucan extract seems consistent owing to its visco-elastic stability, over wide range of frequency sweep. β-glucan extracts having valuable physicochemical as well as rheological characteristics indicates its ability to be incorporated in wide range of food products to maintain their textural and appearance properties along with possessing improved antioxidant characteristics.
The chemical composition such as moisture; protein and crude fiber contents of the flour blends of germinated minor millet were significantly high than the raw minor millets flour blends. The functional properties of flours play an important role in the product development. The water absorption index (WAI) and water solubility index (WSI) decreased in flour blends with increased kodo millet due to decreased protein content. The WAI and WSI of flour blends of germinated minor millet were found significantly ($p \leq 0.05$) higher than the flour blends of raw minor millets. Pasting properties are the important factors in determining the applications of flours. The obtained results of pasting profile revealed that the significant differences were observed in pasting profiles of flour blends of minor millets. As the proportion of kodo millet increased the peak viscosity, breakdown, setback, final viscosity and pasting temperature decreased in flour blends of minor millet from $A_1$ to $A_6$ samples. Analysis revealed that germinated flour blends contained highest protein content, total phenolic content and antioxidant activity and less fat and carbohydrate content than raw flour blend. The flours blend so used could be considered to have potential to reduce protein-energy malnutrition in the developing countries because of the expected improved nutritive value of the blend. Germinated millets may offer the inherent health benefits especially bioactive and related compounds to the consumer. Furthermore the results of this study explore the possible potential utilization of germinated millet grains in food formulations and product development especially health foods.

Gluten free cookies were prepared from minor millet flour (raw and germinated) and the overall results showed that the germinated minor millet flour could be used to prepare cookies with acceptable quality and improved nutrition. The cookies ($A_1$-$A_6$) prepared from flour blends of raw and germinated minor millets were optimized on the basis of physical, textural and sensory properties. Significant differences ($P < 0.05$) were observed in the weight, thickness,
diameter, and spread ratio of the control and the cookies made with the blend flour of raw and germinated minor millet. The diameters of cookies made from raw and germinated minor millet from A1, A2, A3, A4, A5, and A6 were found significantly lower than 100% wheat flour cookies. Cookies made from the blend flour of raw minor millet had higher spread ratio than germinated minor millet blend flour and wheat flour cookies. Cookies having higher spread ratios are considered the most desirable. Significant differences (P < 0.05) were observed in the hardness of the cookies made with the blend flour of raw and germinated minor millet and wheat flour cookies (Table 4). The wheat flour cookies required significant more force to break than cookies of blend flour of raw and germinated minor millet flour. The hardness of cookies of blend flour of raw minor millet was higher than cookies of blend flour of germinated minor millet flour. The blend flour cookies of raw and germinated minor millet were optimized on the basis of physical, textural and sensory properties. Cookies prepared with incorporation raw and germinated foxtail, barnyard and kodo millet in the proportion of 70:20:10 (A2) were found to be acceptable in terms of sensory score. The nutritional analysis of cookies prepared from flour blends of germinated minor millets optimized sample had higher amounts of moisture (7.34 g/100 g); ash (2.78 g/100 g); protein (11.06 g/100 g), dietary fiber (34.36 g/100 g), total phenolic contents (45.43 mg/100) and DPPH radical scavenging activity (64.23%) as compared to raw minor millet flour blended and control cookies. During storage study, it was observed that raw and germinated millet flour based cookies were in acceptable quality up to 120 days at ambient conditions in MET-PPE (metalized polyester polyethylene) packages. The free fatty acid contents for raw flour cookies remained between 0.89 and 1.67 mg KOH/g, while for germinated flour cookies the increase was from 1.06 to 1.78 mg KOH/g. Although there was an increase in free fatty acid content during storage but increase was under permissible limits. The water
activity values indicated that the both raw and germinated flour cookies were stable and safe from microbial spoilage up to 120 days. The highest average over all acceptability was observed for cookies packed in METPPE laminate while the lowest average over all acceptability was observed for raw blend flour cookies and germinated blend flour cookies packed in LDPE.

Cake represents a suitable food product for the addition of functional ingredients, such as the cholesterol lowering dietary fiber minor millets $\beta$-glucan. The incorporation of $\beta$-glucan of minor millet had a positive effect on the nutritional value, textural properties and antioxidant characteristic of wheat based cake. The total dietary fiber and $\beta$-glucan varied from 2.09 to 4.81 g/100 g and 0.47 to 7.99 g/100 g, respectively when the level of $\beta$-glucan was increased from 2.5 to 7.5% in cake. The volume index of cake was increased when the $\beta$-glucan was added in cake up to certain level and decreased at higher levels. Cake favorable factors such as firmness, cohesiveness, springiness, chewiness, were significantly ($p \leq 0.05$) improved in case of cake samples fortified with $\beta$-glucan from 2.5 to 7.5% as compared to control. The decrease in resilience and increase in gumminess and adhesiveness as cake acceptability factors with increase in $\beta$-glucan to 7.5% resulted a decrease in acceptability factor of cake Significant differences were observed ($p \leq 0.05$) in the crumb and crust colors of cake containing $\beta$-glucan extract of germinated foxtail, barnyard and kodo millets. Over all, the brightness ($L$) and yellowness ($b$) of bread crumb and crust was greatly influenced by the level of $\beta$-glucan addition; generally, the $L$ and $b$ values of bread crumbs and crust decreased significantly with increasing $\beta$-glucan level, and $a$ value of crumb and crust increased significantly with increasing $\beta$-glucan levels from 2.5 to 7.5%. Significant differences ($p \leq 0.05$) were observed in antioxidant activity of the control cake and cake containing $\beta$-glucan extracts of germinated foxtail millet, barnyard and kodo millet. The in vitro antioxidant activity varied increased from 18.23 to 22.58% when the
level of β-glucan was increased from 2.5 to 7.5% in cake because the β-glucan extracts of germinated foxtail millet, barnyard and kodo millet. The control cake and cake fortified with 5.0 g β-glucan of minor millet were liked more than the cake fortified with lower or higher levels. FDA approved health claim on a label, adding β-glucan to foods instead of fat/flour is a good way to improve health by increasing dietary fibers and reducing the calories. The incorporation of β-glucan of minor millet has a positive effect on the nutritional value, textural properties and antioxidant characteristic of wheat cake. Control cake and fortified cake with 5.0% β-glucan was in acceptable range up to 8 days at refrigerator conditions. During storage study, it was observed that control and fortified cake with 5.0% β-glucan was in acceptable range up to 8 days at refrigerator conditions. Free fatty acid content was observed to be increased on storage but was in permissible limits. The lowest free fatty acid content was observed on 0 day of storage while the highest free acid content was found on 15 days of storage. The cake fortified with 5.0% β-glucan showed similar (p>0.05) firmness values at 4 and 8 days, but firmness increased after 12 days. The results from microbiological analysis suggest that low storage temperature and addition of β-glucan prolongs the microbiological shelf life of cake.
Future scope of study

- Minor millet grains have immense nutritional potential and nutraceutical properties (higher dietary fiber, TPC, AoxA and GABA), this crop can be commercially grown on a large scale.

- The minor millet grain proteins possesses non-gluten forming property, hence the developed product can be explored at pilot scale for commercialization as gluten free products.

- The studied properties showed that minor millet grains can also be explored as a new source of β-glucan for various food industrial applications.