CHAPTER 2

Review of Literature

2.1 Millet production and importance

In the world, millets are generally grown in India, Africa and China and also they assume significant part in the sustenance of security and economy of numerous developed countries in the world. The initial recorded reports on the development of millets go back to around 5,500 BC in China (Crawford, 2006). They are critical harvests in semi-bone-dry locales where different crops typically don't survive. Millets positions as the 6th most vital grain and nourishes 33% of the aggregate world populace (Saleh et al., 2013). They are easy to cultivate, inherently biodiverse and can be grown together with varied crops (Ahmed et al., 2013). Other qualities of millets that settle on them a favored decision in regions where they are developed are their short collect period (45-65 days) (Bukhari et al., 2011).

In North American and European nations, millets are mostly utilized as a ingredient as a part of composite blends flour, to create gluten free and low glycemic file (GI) nourishment product. Millet food products from 100% millet flour are once in a while produced, however in African and Asian nations, millets serve as the primary ingredient for producing of tradition product and drinks (Saleh et al., 2013). Pearl millet is the most ordinarily expended millet, developed in the parched and semi-bone-dry tropical locales of Asia, Africa and Latin America. India is the biggest maker of pearl millet in Asia and is principally developed in northwestern parts (Obilana, 2003). It is likewise the pearl millet developed in Nepal and Bhutan (Mal et al., 2010). China nonetheless, principally produces foxtail millet. Finger millet is developed in more than 25 nations in eastern and southern Africa, and crosswise over Asia, with the real makers being Uganda, India, Nepal and China. In Africa, pearl millet creation is amassed in Sahara and
drier territories of northern and eastern Africa (ICRISAT, 1996; Obilana, 2003). Millets differ from one another by their appearances, and morphological features, maturity, grain type, etc. (Figure 2.1).
Barnyard millet  

kodo millet  

Foxtail millet  

Proso millet  

Little millet

Figure 2.1 Pictures of commonly used Millet
2.2 Nutritional composition of millets

Millets are significantly rich in resistant starch, soluble and insoluble dietary fibers, minerals, and antioxidants. It contains about 92.5% dry matter, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali et al., 2003). Black finger millet contains 8.71 mg/g dry weight fatty acid and 8.47 g/g dry weight protein (Glew et al., 2008). Kodo millet and little millet were also reported to have 37% to 38% of dietary fiber, which is the highest among the cereals; and the fat has higher polyunsaturated fatty acids (PUFA) (Hegde and Chandra, 2005). Kalinova and Moudry (2006) reported the protein content of proso millet about 11.6% being rich in essential amino acids (leucine, isoleucine, and methionine). The average of nutrient composition of some millet grains and other grains is summarized in Table 2.1.

2.3 Bioactive compounds of millet

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 (Goldberg, 2003). Nutraceuticals are natural bioactive compounds that have health promoting, disease preventing or medicinal properties. Bioactive compounds are extra nutritional elements that typically occur in small quantities in foods. These substances are beneficial to human health but are not essential for the human body (Kris-Etherton et al., 2002). Millets must also be accepted a functional food and nutraceuticals because they provide dietary fibers, proteins, energy, minerals, vitamins, and antioxidants required for human health. The majority of bioactive compounds of whole-millet grains are present in the bran/germ fraction of cereal-grains. Several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol,
and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk were reported for millets (Gupta et al., 2012).

Table-2.1 Nutrient composition of major cereals and millets (per 100 g of edible portion at 12% moisture

<table>
<thead>
<tr>
<th>Cereals</th>
<th>Energy (kcal)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>CF (g)</th>
<th>Calcium (mg)</th>
<th>Iron (mg)</th>
<th>Niacin (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (brown)</td>
<td>362</td>
<td>76.0</td>
<td>7.9</td>
<td>2.7</td>
<td>1.0</td>
<td>33.0</td>
<td>1.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Wheat</td>
<td>348</td>
<td>71.0</td>
<td>11.6</td>
<td>2.0</td>
<td>2.0</td>
<td>30.0</td>
<td>3.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Maize</td>
<td>358</td>
<td>73.0</td>
<td>9.2</td>
<td>4.6</td>
<td>2.8</td>
<td>26.0</td>
<td>2.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Sorghum</td>
<td>329</td>
<td>70.7</td>
<td>10.4</td>
<td>3.1</td>
<td>2.0</td>
<td>25.0</td>
<td>5.4</td>
<td>4.3</td>
</tr>
<tr>
<td>Pearl millet</td>
<td>363</td>
<td>67.0</td>
<td>11.8</td>
<td>4.8</td>
<td>2.3</td>
<td>42.0</td>
<td>11.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Finger millet</td>
<td>336</td>
<td>72.6</td>
<td>7.7</td>
<td>1.5</td>
<td>3.6</td>
<td>350</td>
<td>3.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Foxtail millet</td>
<td>351</td>
<td>63.2</td>
<td>11.2</td>
<td>4.0</td>
<td>6.7</td>
<td>31.0</td>
<td>2.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Proso millet</td>
<td>364</td>
<td>63.8</td>
<td>12.5</td>
<td>3.5</td>
<td>5.2</td>
<td>8.0</td>
<td>2.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Little millet</td>
<td>329</td>
<td>60.9</td>
<td>9.7</td>
<td>5.2</td>
<td>7.6</td>
<td>17.0</td>
<td>9.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Kodo millet</td>
<td>353</td>
<td>66.6</td>
<td>9.8</td>
<td>3.6</td>
<td>5.2</td>
<td>35.0</td>
<td>1.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Abbreviations: CHO, carbohydrates; CF, crude fibre. (Source: data adapted from reference FAO, 1995).

Chandrasekara and Shahidi (2011a) reported the content of a number of flavonoids, namely flavan-3-ols (monomers, and dimmers), flavonols and their glycosides, flavones and flavononol, in millet phenolic extracts mainly in the soluble fraction (Table-2.2). Issoufou amadoul et al., (2013) reported that the millets are source of antioxidants, such as phenolic acids.
and glycated flavonoids and the nutraceuticals. Millet foods are characterized to be potential prebiotic and can enhance the viability or functionality of probiotics with significant health benefits. Processing of millet by milling removes the bran and germ layers that are rich in fiber and phytochemicals, causing significant loss. The nutritional significance of millet demands an examination of the nutritional characteristics and functional properties of different millet cultivars as well as developing value added products from millets.

Chaturved et al. (2011) studied that the products of wheat rice, millets, barley, oat, buckwheat, corn, sorghum, flaxseed, psyllium and brown rice are to notify the most common cereal based functional foods and nutraceuticals. The nutrients in the cereals have been identified prospective for reducing the risk of coronary heart disease, diabetes, tumor incidence, cancer risk, blood pressure, reduces the rate of cholesterol and fat absorption, delaying gastrointestinal emptying and providing gastrointestinal health. The regular insertion of cereals and their processed products can make a payment to health endorsement and disease avoidance. Sandeep et al., (2014) has carried out the proximate and phytochemical analysis of the seed coat of minor millet where in, the reducing sugar content, total carbohydrate, elemental analysis, moisture content, total fat, total protein, total fiber content and total ash were estimated.

Rao et al., (2011) reported that the kodo millet has maximum phenolic content (10.3%) and crude fiber content (14.3%) while foxtail millet has shown minimum phenolics (2.5%). As far as reducing capacity of free radicals were concerned, finger millet showed highest (5.7%) and proso millet showed least reducing property (2.6%).
## Table 2.2 Major phenolic acids and flavonoids in millet grains

<table>
<thead>
<tr>
<th>Millet</th>
<th>HBAS</th>
<th>HCAS</th>
<th>FLOS</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl</td>
<td>p-Hydroxybenzoic, protocatechueic, vanillic</td>
<td>Caffeic , p-coumaric, cinnamic, sinapic c,tr ans -ferulic</td>
<td>Apigenin, myricetin</td>
<td>Chandrasekara and Shahidi (2011c), N’Dri et al. (2012)</td>
</tr>
<tr>
<td>Foxtail</td>
<td>Gallic, p-hydroxybenzoic, protocatechueic, syring ic, gentisic, vanillic</td>
<td>Caffeic , p-coumaric, sinapic, tr ans -ferulic</td>
<td>Catechin, quercetin, apigenin, kempherol</td>
<td>Chandrasekara and Shahidi (2011c)</td>
</tr>
<tr>
<td>Proso</td>
<td>Gallic,p-Hydroxybenzoic, protocatechueic, syring ic, gentisic, vanillic</td>
<td>Chlorogen ic, caffeic, p-coumaric, sinapic, ferulic</td>
<td>Kempherol, apigenin, Myricetin</td>
<td>Chandrasekara and Shahidi (2011c)</td>
</tr>
<tr>
<td>Kodo</td>
<td>Gallic,p-hydroxybenzoic, protocatechueic, syring ic, vanillic</td>
<td>Chlorogen ic, caffeic, p-coumaric, sinapic c,tr ans -ferulic</td>
<td>Kempherol, apigenin, vitexin, isov itexin, luteolin, quercetin</td>
<td>Chandrasekara and Shahidi (2011c)</td>
</tr>
<tr>
<td>Little</td>
<td>Gallic, protocatechueic, syring ic, gentisic, vanillic</td>
<td>Caffeic , p-coumaric, sinapic c,tr ans –ferulic</td>
<td>Apigenin</td>
<td>Chandrasekara and Shahidi (2011c)</td>
</tr>
<tr>
<td>Barnyard</td>
<td>Na</td>
<td></td>
<td>Luteolin, tricin, N-(p-coumaroyl) serotonin</td>
<td>Watanabe (1999)</td>
</tr>
</tbody>
</table>

Abbreviations: na, data not available; HABS, hydroxybenzoic acids and their derivatives; HACS, hydroxycinnamic acids and their derivatives; FLOS, flavonoids

Pradeep and Guha (2015) reported that the total phenolic, flavonoid and tannin contents of processed little millet increased by 21.2, 25.5 and 18.9 mg/100 g, respectively, compared to
native sample. The iron reducing power and the of roasted millet extract were the DPPH radical scavenging activity highest compared to the other processed millet. Raw barnyard millet possessed highest TPC (80.14 ± 0.57 mg FAE/100 g) followed by proso (74.43 ± 1.15 FAE/100 g) and foxtail (72.70 ± 2.29 mg FAE/100 g) millets. Some researchers reported that the phenolic acids such as p-hydroxybenzoic, vanillic, ferulic, sinapic and p-coumaric acids are also reported in the crude extract of proso millet) and presence of free aglycones, apigenin, naringenin and kaempferol in finger millet. Luteolin and tricin are reported in Japanese barnyard millet (*Echinochloa crus-galli* (L.) (Kim *et al.*, 2013; Shobana *et al.*,2009). (Watanabe, 1999). The apigenin content of proso millet (15.53 ± 1.34 lg/g) in the higher than foxtail millet (101.96 ± 1.92 lg/g) lg/g) Chandrasekara and Shahidi (2011).

### 2.4 Potential health benefits of millet grains

Researchers have revealed that diets containing plant or whole grain materials are beneficial in the prevention of several chronic diseases like cancer, cardiovascular ailments, diabetes, metabolic syndrome, and Parkinson’s disease and also act against age-related diseases such as diabetes, cardiovascular and carcinogenic diseases (Manach *et al.*, 2005; Scalbert *et al.*, 2005; Chandrasekara and Shahidi, 2012). This could be attributed to the presence of vitamins, minerals, essential fatty acids, bioactive compounds and fiber in whole grains that are found to have positive health benefits. Besides the nutritional components, they are rich sources of resistant starch, oligosaccharides and photochemical. Millets are the rich sources of proteins, minerals, vitamins, dietary fibre and possess antioxidant compounds including phenols and flavonoids, which have been reported to protect the human body from carcinogenic and cardiovascular diseases, tumors, increasing blood pressure, rise in cholesterol level, and lowering of fat absorption (Truswell, 2002; Gupta *et al.*, 2012).
2.4.1 Antioxidants and their activities

Antioxidant compounds are gaining importance due to their main roles as lipid stabilizers and as suppressors of excessive oxidation that causes cancer and ageing (Namikii, 1990). Their stable radical intermediates prevent the oxidation of various food ingredients, particularly fatty acids and oils (Maillard et al., 1996). Phenolic acids and their derivatives, flavonoids and tannins present in millets seed coat are having multifunctional characteristics and can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers (Sripriya et al., 1996). The potency of phenolic compounds to act as antioxidants arise from their ability to donate hydrogen atoms via hydroxyl groups on benzene rings to electron deficient free radicals and in turn form a resonance-stabilized and less reactive phenoxy radical. Studies were carried out on the natural antioxidants in edible flours of small millets. Total antioxidant capacity of finger, little, foxtail and proso millets were found to be higher and their total carotenoids content varied from 78–366 mg/100 g in the millet varieties.

A number of research studies are evaluated the antioxidant activity of phenolics and other bioactive components extracted from millet grains. (Watanabe, 1999) reported that 3 antioxidative phenolic compounds, 1 serotonin derivative, and 2 flavonoids are isolated from an ethanol extract of Japanese barnyard millet grains. Their structures were established to be \( N-(p\text{-coumaroyl}) \) serotonin, luteolin, and tricin. Although the antioxidant activity of luteolin was lower than that of \( N-(p\text{-coumaroyl}) \) serotonin, it was nearly equal to that of quercetin, whereas the activity of tricin was lower than that of luteolin. Kodo millet, finger millet, little millet, foxtail millet, barnyard millet, and sorghum \( bicolor \) grown in India and their white varieties were screened for free radical quenching of 1,1, diphenyl-2-picrylhydrazyl (DPPH) by electron spin resonance. Methanol extracts of the kodo millet flour showed 70% DPPH quenching in
comparison to other millet extracts that showed 15% to 53%. Hegde and Chandra (2005) have been also reported that the white varieties of sorghum, finger millet, and foxtail millet showes lower quenching than their colored counterparts, indicating that phenolics in the seed coat could be responsible for the antioxidant activities. Furthermore, finger millet extracts were found to have a potent radical-scavenging activity that is higher than those of wheat, rice, and other species of millet (Dykes and Rooney, 2006). The reducing power of finger millet seed coat extract is significantly ($P < 0.05$) higher than that of whole flour extract (Viswanath et al., 2009). Moreover, Veenashri and Muralikrishna (2011) observed that xylo-oligosaccharides (XOs) mixture of finger millet exhibited relatively higher antioxidant activity than the XOs of rice, wheat, and maize by DPPH and ferric reducing antioxidant power assays. Suma and Urooj (2011) indicated that foxtail millet, methanolic extracts of whole flour and bran-rich fraction exhibited a significantly higher ($P < 0.05$) radical-scavenging activity (44.62% and 51.80%, respectively) using a DPPH model system, and reducing power (0.381 and 0.455, respectively) at 2 mg, than the ethanol and water used for extraction. On the other hand, some researcher 50% ethanol extract from defatted foxtail millet bran is found to be the best-promoting phenolic compound with substantial antioxidant activity and defatted foxtail millet protein hydrolysates also exhibited antioxidant potency (Amadou et al., 2011; Mohamed et al., 2012). Thus, millet may serve as a natural source of antioxidants in food applications and as a nutraceutical and functional food ingredient in health promotion and disease risk reduction. However, more studies in animal models and with human subjects should be performed to verify their activity and health benefits.
2.4.2 Millet for diabetics

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia with alterations in carbohydrate, protein, and lipid metabolism. Reports indicate that hyperglycemia can induce non-enzymatic glycosylation of various proteins, resulting in the development of chronic complications in diabetes (Lebovitz, 2001). Therefore, control of postprandial blood glucose surge is critical for treatment of diabetes and for reducing chronic vascular complications (Barvo, 1998) which can be controlled by intake of high complex carbohydrate and high fiber diet. For example, consumption of finger-millet based diets results in significantly lowering plasma glucose levels that might have been due to the higher fiber content of finger millet than rice and wheat. Gopalan (1981) reported that regular consumption of finger millet is known to reduce the risk of diabetes mellitus and gastrointestinal tract disorders. In another study, phenolic compounds from the millets seed coat showed strong inhibition toward α-glucosidase and pancreatic amylase. The beneficial effect of phenolics is due to partial inhibition of amylase and α-glucosidase during enzymatic hydrolysis of complex carbohydrates and delays the absorption of glucose, which ultimately controls the postprandial blood glucose levels (Shobana et al., 2009). Therefore, millet grains have the potentials to be useful in preventing diabetes and for the treatment of diabetics.

2.4.3 Millet against cardiovascular disease

Most of the world countries face high and increasing rates of cardiovascular disease. It has been demonstrated that rats fed with a diet of native and treated starch from barnyard millet had the lowest blood glucose, serum cholesterol, and triglycerides as compared with rice and other minor millets (Kumari and Thayumanavan, 1997). Also, the feeding of proso millet protein improves the plasma levels of adiponectin, high-density lipoprotein (HDL) cholesterol in
genetically obese type-2 diabetic mice under high-fat feeding conditions (Park et al., 2008). Finger millet and proso millet may prevent cardiovascular disease by reducing plasma triglycerides in hyperlipidemic rats (Lee et al., 2010). In addition, phenolic extracts from kodo, finger, proso, foxtail, little, and pearl millets were evaluated for their inhibitory effects on lipid peroxidation in vitro copper-mediated human LDL cholesterol oxidation and several food model systems, namely, cooked comminuted pork, stripped corn oil, and a linoleic acid emulsion. At a final concentration of 0.05 mg/ml, millet extracts inhibit LDL cholesterol oxidation by 1% to 41%. Millets also exhibit effective inhibition of lipid oxidation in food systems (Chandrasekara and Shahidi 2011b).

2.4.4 Millets against cancers

Millet grains based on literature values are known to be rich in phenolic acids, tannins, and phytate that act as “anti-nutrients” (Thompson, 1993). However, it has been established that these antinutrients reduce the risk of colon and breast cancer in animals (Graf and Eaton, 1990). It has also been reported that populations consuming sorghum and millets have lower incidences of esophageal cancer than those consuming wheat or maize (Van Rensburg, 1981). Furthermore, a recent study has demonstrated that millet phenolics may be effective in the prevention of cancer initiation (Chandrasekara and Shahidi, 2011c).

2.4.5 Millets against celiac disease

The overall growing demand for novel, tasty, and “healthy”, foods, together with the increasing number of people suffering from celiac disease, has given birth to a new market consisting of cereal products made from grains other than wheat and rye. In this challenging market, oat, sorghum, and millet have gained a special position (Angioloni and Collar, 2012a). Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten in
genetically susceptible individuals. It is one of the most common lifelong disorders worldwide. In the past, celiac disease was considered a rare disorder, mostly affecting children of European origin. In the developed countries, there is a growing demand for gluten-free foods and beverages from people with celiac disease and other intolerances to wheat, barley, or rye. However, millets are gluten-free; they have considerable potential in foods and beverages that can be suitable for individuals suffering from celiac disease. Therefore, millet grains and their fractions have the potential to be useful for producing food products for celiac people (Chandrasekara and Shahidi, 2011b).

2.4.6 Antimicrobial activity of millets

Baranowski et al. (1980) have reported that phenolics have been implicated for minimising the intensity of several diseases and also to inhibit the in vitro growth of an assortment of fungal genera. Millet grain extracts were found to have antimicrobial activity. In one study, seed protein extracts of pearl millet, sorghum, Japanese barnyard millet, foxtail millet, samai millet, and proso millet were evaluated in vitro for their ability to inhibit the growth of Rhizoctonia solani, Macrophomina phaseolina, and Fusarium oxysporum. In another study, phenolic acids from finger millet milled fractions (whole flour, seed coat, 3%, 5%, and 7%) were isolated. The seed coat extract of millet showes higher antimicrobial activity against Bacillus cereus and Aspergillus flavus than whole flour extract. The results indicated that potential exists to utilize finger millet seed coat as an alternative natural antioxidant and food preservative (Viswanath et al., 2009). Therefore, extracts of phenolic acids and other bioactive components have the potential to be used as natural alternatives in food preservation and for therapeutic purposes.
2.4.7 Millet and aging

The chemical reaction between the aldehyde group of reducing sugars and the amino group of proteins, termed as non enzymatic glycosylation, is a major factor responsible for the complications of diabetes and aging (Monnier, 1990). Millet grains are rich in antioxidants and phenolics; however, it has been established that phytates, phenols, and tannins can contribute to antioxidant activity important in health, aging, and metabolic syndrome (Bravo, 1998). It has also been found that methanolic extracts from finger and kodo millets inhibited glycation and cross-linking of collagen (Hegde et al., 2002). Therefore, there is potential usefulness of millets in the protection against aging.

2.4.8 Health benefits of millet beta-glucan

Minor millet is the new source of $\beta$-glucans is derived from cell wall of grains. The edible outermost layer of the millet kernel is rich in $\beta$-glucans. They also occur in plant cellulose, the bran of cereal grains, cell wall of fungi, mushrooms, bacteria, sea breams and algae. $B$, 1-3 and 1-4 - glucans are extracted from the bran of the some grains such as oats, barley, rye and wheat. The $\beta$-(1, 3) glucans from yeast cell wall are insoluble, whereas from grains are both soluble and insoluble. It is a natural polymer comprised of individual glucose molecules that are linked together by a series of $\beta$-(1, 3) and $\beta$-(1, 4) linkages, comprising a class of non digestible polysaccharides called $\beta$- glucans. This unique array of linking promotes several consumer health benefits.

2.4.8.1 $\beta$-glucan role in the treatment of infectious diseases

Luzio and Riggi (1970) studied the impact of $\beta$-glucan for reducing the incidence of infection with high-risk surgical patients. Some researcher studied that cell wall $\beta$-glucans were identified as the active component, having various effects on the immune system such as anti-
tumor activity as well as anti-infective activities that include protection against fungal, bacterial, viral, parasitic and protozoan infections (Itoh, et al., 1997; Williams, 1997)

2.4.8.2 β-glucan role in the control of cholesterol and diabetes

β-glucans are effective hypoglycemic and hypocholesterolemic agents in human diets and reduce serum cholesterol level and attenuate post brandial blood glucose and insulin response in a viscosity related fashion (Newman,1989; Braaten et al.,1994). Oat β-glucans increase bile acid excretion by inhibiting bile acid reabsorption. Viriyakosal et al. (2005) reported that β-glucans reduces the risk of cardiovascular disease by lowering serum cholesterol and the mediating effect on stabilizing blood glucose and insulin levels in diabetes. Yeast β-glucans appear to be effective in lowering blood cholesterol but the mechanism is still unclear. Several fungal β-glucan reduce blood glucose level after eating, possibly by delaying stomach emptying so that dietary glucose is absorbed more gradually (Li et al., 2006).

2.4.8.3 β-glucan role in treatment of blood pressure, radiation exposure, septic shock and surgery

β-glucan reduces the production of pro-inflammatory cytokines, mainly tumor necrosis factor alpha which reduced mortality (Soltes et al.,1993). Yeast β-glucan reduces septic shock by killing bacteria in blood. Kaiser et al 1998 reported that preventive dosing of yeast glucan prior to infection with S. aureus prevented sepsis in guinea pig (Kaiser and Kernodle, 1998). β-glucan protects against oxidative organ injury

2.4.8.4 β-glucan role in treatment of cancer

Orally delivered glucan found to increase proliferation and activation of monocytes in peripheral blood of patients with advanced breast cancer (Wood, 2007). Intralesional administration of β-glucans resulted in rapid tumor shrinkage delivered yeast β-glucan with
monoclonal antibody therapy increased neuroblastoma tumor regression and long term survival in mice (Liu et al., 2009). Harada et al. (1966) reported that some β-glucans ameliorate chemotherapy and radiation treatment by increasing patient tolerance and speed recovery from toxic effects.

2.4.9 Health benefits of γ-amino butyric acid (GABA)

Komatsuzaki et al. (2007) indicated that γ–amino butyric acid (GABA) is a four carbon amino acid that is produced by the decarboxylation of L-glutamic acid that catalyzed by glutamate decarboxylase enzyme GABA is an inhibitory neurotransmitter in the sympathetic nervous system (Wang et al., 2006). Germinated cereal grains have been widely studied for GABA production such as in germinated barley (Chung et al., 2009), germinated rice (Zhang et al., 2007; Thuwapanichayanan et al., 2015; Kim et al., 2015). GABA provides many beneficial effects for human health.

2.4.9.1 γ-amino butyric acid (GABA) role in the treatment of depression

Krystal (2002) studied that the role of GABA for depression has been sparked by preclinical studies suggesting GABA levels are decreased in patients suffering from depression. Various antidepressant drugs have been shown to be effective for depression by affecting not only monoamine and serotonin activity, but the role of GABAergic dysfunction in mood disorders was first proposed (Shiah and Yatham, 1998; Brambilla et al., 2003).

2.4.9.2 γ-amino butyric acid (GABA) role in the treatment of sleep enhancement

Due to its relaxation effects, GABA may be considered as a sleep aid. GABAA receptors are highly expressed in the thalamus, a region of the brain involved with sleep processes Orser (2006). Some researcher observed that GABA-agonist drugs, such as zolpidem (Ambien) and temazepam (Restoril), are sedatives used in the treatment of insomnia (Palagini et al., 2016; Roth
et al., 2006). The synthetic GABA-like drug gabapentin that increases brain GABA levels has been found to improve sleep disturbances associated with alcohol consumption (Bazil, Battista and Basner, 2005).

2.4.9.3 γ-amino butyric acid (GABA) role in the treatment of epilepsy

The mechanisms of most anti-epileptic drugs involve direct or indirect GABA enhancement. The drugs act in a variety of ways by increasing GABAergic inhibition (benzodiazepines, phenobarbital, valproate), inhibiting GABA reuptake (tiagabine), increasing synaptic GABA concentration through inhibition of gamma-amino butyrate transaminase (vigabatrin), and increasing brain synaptic GABA and decreasing neuronal influx of calcium ions (gabapentin) (Linazasoro, and Van Blercom, 2007)

2.4.9.4 γ-amino butyric acid (GABA) role in the treatment of movement disorders: tourette syndrome, parkinson’s disease, tardive, dyskinesia

As the main inhibitory neurotransmitter, it is not surprising GABAergic pathways are involved in the pathophysiology of various movement disorders. Go et al. (2001) reported that baclofen is a synthetic GABA analogue exerts antispasmodic effect and gabapentin have been found to benefit patients with Parkinson’s disease, while the GABA agonist vigabatrin (gamma-vinyl-GABA) provides benefit for tardive, dyskinesia and other movement disorders (Awaad, 1999; Chen and Li, 2007).

2.5 Effect of bio-processing on nutritional profile and functional ingredients

Malting is a controlled germination process which activates the enzymes of the resting grain resulting in the conversion of starch to fermentable sugars, partial hydrolysis of proteins and other macromolecules (Ravindran et al., 1995). It is recognized that simple traditional food processing treatments, like soaking and malting/germination may significantly reduce the anti-
nutrient contents of cereal grains and improve their nutrients’ bio-availabilities (Najdi et al., 2016). Germination is the most common and effective process to improve the quality of cereal grain. Soaking is the first step in water penetration, which transforms the inactive tissue into living tissue. In this step, the grain’s metabolism is activated in preparation for germination. During germination, the grain nutrient reserves degrade and are used for respiration and the synthesis of new cells that form the developing embryo, causing changes in the nutritional and biochemical compositions (Bamforth and Barclay, 1993).

2.5.1 Soaking

Soaking is a simple technological treatment that is often used by mothers to prepare complementary foods at home. Moreover, it can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds (Lestienne et al., 2005). Previous studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and to an enhancement of mineral HCl-extractability, used to estimate mineral bioavailability (Duhan et al., 2002). Lestienne et al., (2005) observed that depending on the botanical origin of the seeds, a significant reduction in phytate content (between 17% and 28%) is obtained by soaking whole seeds for 24 h at 30°C. (Lestienne et al., 2005) observed that depending on the botanical origin of the seeds, a significant reduction in phytate content (between 17% and 28%) is obtained by soaking whole seeds for 24 h at 30°C. Insignificant loss of total free phenolics in V. aconitifolia has been noticed while soaking in 13% distilled water (Vijayakumari et al., 1998). Vijayakumari et al. (1998) also reported that tannin content of V. aconitifolia gets reduced significantly by either distilled water or NaHCO₃ solution soaking.
The increased levels of GABA during the soaking treatment resulted from GmGAD1 protein synthesized in developing soybean seeds, and this implies that GmGAD1 mRNA expression is not the only factor to increase GABA in the early period of germination-treated soybean seeds or soaking-treated soybean seeds according to the researches of Matsuyama et al. (2009).

2.5.2 Germination/malting

Germination/malting of the grain change the chemical composition, nutritive value, and acceptability characteristics of products for human consumption. During the process of germination a significant changes in the biochemical, nutritional and sensory characteristics of cereals occur due to degradation of reserve materials as used for respiration and synthesis of new cell constituents for developing embryo in the seed (Danisova et al., 1995). It was found that stacchyose and raffinose, generally attributed to flatulence, decrease during the germination process. The caloric content of the seed decreases during the germination but the nutrient-energy ratio of some vitamins goes higher than the original seed (Jaya and Venkataraman, 1981). The germination process improves the nutritional quality of cereals and legume.

Germinated legumes are widely consumed all around the world (Ridge, 1991). Rao et al. (2004) shows that finger millet can be incorporated as a source of dietary fiber both in the native and malted forms, in the preparation of various health foods without altering the dough characteristics or the quality of the end product. Therefore, malting generally improves the nutrient content and digestibility of foods and it could be an appropriate food-based strategy to derive iron and other minerals maximally from food grains (Platel et al., 2010). Inyang and Zakari (2008) observed that effect of germination and fermentation of pearl millet on proximate, chemical, and sensory properties of instant fura (a Nigerian cereal food) and it is found that
germination appears to be a promising food processing method for improving the nutrient and energy densities of *fura* and, when combined with fermentation, reduced phytic acid significantly ($p<0.05$). Therefore, germination of millet grains can be used as a technique or in combination with other processing treatments to prepare malt rich in nutrients that can be used for the preparation of several healthy and nutritional food products such as infant formula, complementary food products, and composite flours or food blends. However, there is a need for the application of malting at an industrial scale using novel germinators enhanced by a control system of germination conditions to provide high-quality malt products that can be easy to handle and consumed by larger populations to help in promoting millet utilization.

2.5.2.1 Effect of malting/germination on nutritional value

Germination or malting of cereal grains may result in some biochemical modifications and produce malt with improved nutritional quality that can be used in various traditional recipes. It has been found that germination of proso millet grains increases the free amino acids and total sugars and decreases the dry weight and starch content. Increases in lysine, tryptophan, and non-protein nitrogen are also noticed by Parameswaran and Sadasivam (1994). As compared to un-germinated seed, germinated seeds contain high protein, low unsaturated fatty acids, low carbohydrate (Narsih and Wignyanto, 2012). Mineral content such as phospour, calcium, zinc and copper were reported increases in sprouts as the hydrolysis of phytic acid by the phytic enzyme activated during germination (Grewal and Jood, 2006).

Some researcher have reported that soaking, germination, debraning and dry heating can be attributed to the reduction of antinutrients such as phytic acid, tannins, and polyphenols, which are known to interact with proteins to form complexes as a result the *in vitro* digestibility of protein increases (Hassan *et al.*, 2006). El-Hag *et al.* (1978) found that combination of
sprouting and cooking results in a better digestibility of protein in legumes. Mineral value increases during the germination process. Iron availability increases to significant level due to increase in phytase activity during germination (Walker and Kochhar, 1982). However, Choudhury et al. (2011) found that after the germination, crude protein and fat contents gets reduced in foxtail millet attributed to loss of low molecular weight nitrogenous compounds during soaking and rinsing of the millet grains and hydrolysis of lipid and oxidation of fatty acids during germination. Also, the changes in nutrient contents of grains after germination can be attributed to the utilization by growing sprouts (Hooda and Jood, 2003).

The ant-nutritional factors such as phytic acid gets decreased in finger and pearl millets during germination, however, the in vitro extractability and bioaccessibility of minerals such as calcium, iron, and zinc gets increased (Mamiro et al., 2001; Suma and Urooj, 2011b; Krishnan et al., 2012). Eyzaguirre et al. (2006) observed that the relative in vitro solubility of iron doubles by the germination of pearl millet grains. In terms of its potential for lager beer brewing, pearl millet malt is reported to have some advantages compared to sorghum since it has higher beta-amylase activity and higher free $\alpha$-amino nitrogen (Pelembe et al., 2004). Furthermore, Grewal and Jood (2006) reported that antinutrients like polyphenols and saponins are also known to hinder the availability of minerals, but after the germination due to increase in phytase activity results in decrease in the content of phytate in sprouts which enhance the mineral availability. Germination of foxtail millet for 3 days results in flour with a high concentration of minerals (Coulibaly and Chen, 2011).

Finger millet could be used as a source of dietary fiber in both native and malted forms for the preparation of several functional foods without any deterioration of final product (Rao et al., 2004). Malting of millets had been reported to enhance the nutritional value along with
digestibility of foods, besides improving the retention of iron and other minerals in cereal based food products (Platel et al., 2010). The process of germination and fermentation of pearl millet results in increasing the nutrients, sensory characteristics and energy value in *fura* (a Nigerian cereal food), as it leads to reduction of phytic acid significantly, (Inyang and Zakari, 2008).

Germinated and fermented pearl millet-based food products has been found to maintain cell viability adequately in comparison to non-germinated millet blended foods and also causes the improvement in the contents of thiamine, niacin, total lysine, protein fractions, sugars, soluble dietary fiber, and *in vitro* availability of Ca, Fe, and Zn of millet blended food products (Arora et al., 2011). Germination can thus be used as a processing parameter along with other processing operations in the preparation of malt that is a rich source of nutrients and finds application in the preparation of numerous functional and nutritional foods like infant formula, complementary food products, and composite flours or food blends. It is thus clear that the process of malting at an industrial scale by means of scientific techniques of germination could accelerate the utilization of millets as a functional food for the ever increasing population demand of nutrients at reasonable rates.

### 2.5.2.2 Effect of germination on bioactive compounds of millet

The consumption of sprouts can be very important in reducing the human diseases related to oxidative stress due to the presence of antioxidants. In the functional foods, the sprouts act as common active ingredients which play an important role in the prevention of oxidation and cellular damage by inhibiting or delaying the oxidative process (Pasko et al., 2009; Silva et al., 2013). The nutraceuticals quality of cereals, pseudocereals, and legumes has been improved by the process of germination. During this bioprocess, some compounds with antioxidant activity increase, mainly polyphenols and flavonoids, which provide protection against oxidative damage.
(Hübner and Arendt, 2013; Randhir et al., 2004; Alvarez-Jubete et al., 2010). Pradeep et al., (2011) have studied the effect of germination, steaming and roasting on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*). The results show that the total phenolic, flavonoid and tannin contents of processed little millet increases by 21.2, 25.5 and 18.9 mg/100 g, respectively, compared to native sample. The DPPH radical scavenging activity and the iron reducing power of roasted millet extract being the highest as compared to the other processed millet.

Mohankumar et al., (2013) studied the effect of different processing method (boiling, blanching, soaking, and germination) on nutrients composition of minor millet. It has been observed that the soaked samples of foxtail and proso millet has shows higher scavenging activity which is found to be (51.06% and 52.12%) respectively. It has further been reported that the antioxidant power of foxtail millet and proso millet increases significantly during germination (317.5 mol and 236.8 mol) respectively. Donkor et al., (2012) observed that germinated rye contains higher amounts of total phenolics content as compared to the non-germinated rye. Radical scavenging activities of the phenolic extracts range between 13% and 73% for non-germinated rye and 14% and 53% for germinated rye.

Besides the above said nutrients germination process increases bio functional component like GABA (γ-aminobutyric acid). The GABA content increased 16.7 folds, after germination. This indicates that introducing a germination process was successful in terms of increasing this bio-active compound in brown rice (Banchuen et al., 2009). GABA (γ-aminobutyric acid) currently is an interesting compound which increases during germination via protein metabolism of seed components (Park et al., 1999). Shiahs and Yatham (1998) have reported that the γ-amino butyric acid directly affects the personality and the capability of a person to manage stress; It
also acts as a neurotransmitter in the brain. The Qingyun et al., (2008) reported that during the entire 60 h germinating period, GABA yields in germinated foxtail millet increases by 2.21 folds in citrate solution and 1.81 folds in acetate buffer solution. The results show that 12 h of soaking at pH 7, followed by 36 h of germination was the optimum condition to achieve maximum GABA content (0.2029 mg/g of germinated BR (GBR)). Provided that all these conditions are met, the model predicted that a maximal content of GABA increases to 2.60 mg/g (db). In our previous publication, some researcher has investigated the effects of culture conditions on GABA accumulation during germination of foxtail millet and fava beans under hypoxia treatments. Komatsuzaki et al. (2007) observed that the GABA accumulation 0.249 mg/g DW and 2.65 mg/g DW in germinated brown rice and soybean respectively under soaking and gaseous treatment. The GABA content in Koshihikari germinated brown rice increased during germination and after 72 h was 11.5 times higher than brown rice. Results show that GABA content increased steadily from 3.96 mg/100 g dry matter at 0 hr duration (i.e., no germination period) to 10.04 mg/100 g dry matter after 12 h, reaching the highest levels of 17.87 mg/100 g dry matter at 24 hr incubation, and then decreased continuously afterwards to 9.91 and 1.36 mg/100 g dry matter at 36 and 48 hr, respectively (Karlaede and Suriyong, 2012).

Similarly, the other functional ingredient, β-glucan dietary fibre intake, which is the main fraction in of endosperm cell wall (Cui and Wang, 2008) has a number of health benefit such as it possess a specific antioxidant capacity which causes a decrease in serum cholesterol levels in humans (Kofuji et al., 2012). Cui et al. (2005) also indicated that β-glucan obtained from mushrooms act as biological response modifier (BRM) which is used in both modern medicine and traditional chemotherapeutic drug for the treatment of various infectious diseases and cancer. β-glucan are part of the endosperm cell wall (Cui and Wang, 2008) and are degraded during the
germination process and result in very low contents of $\beta$-glucan in barley malts as well as oat malts (Belitz, Grosch and Schieberle, 2001). Temelli, Bansema and Stobbe (2004) reported that the use of $\beta$-glucan as functional ingredient for beverages has been investigated and (Brennan and Cleary, 2005) possibilities for the use as ingredient for functional food. Germination process for oats with a high retention of beta glucan. During germination, beta-glucan is quickly degraded in both cereals. Un-malted barley contained 3.8% beta-glucan whereas un-malted oats 4.2% (Hübner et al., 2010).

2.5.2.3 Effect of germination on the antinutritional factor

Polycarpe Kayodé et al., (2013) studied that during the germination process, the tannin content of the sorghum decreases from 429.5 to 174.1 mg/100 g dry matter, while the total phenolic content increased from 300.3 to 371.5 mg GAE/100 g. Phytate content of sorghum grain decreased drastically from 1003 to 369.1 mg/100 g dry matter when the duration of germination or fermentation increases. The antinutritional factors like trypsin inhibitory activity, phytates, tannins and total polyphenols reduces to considerable amount after germination (Ramakrishna et al., 2006). Chove and Mamiro, (2010) observed that during germination cyanide content increased where as autoclaving process resulted decrease in cyanide levels in the finger millet. It was found that germination increased the cyanide content by 2.11 to 2.14 fold in finger millet and autoclaving reduced the cyanide content to between 61.8 and 65.9 % of the original raw contents for finger millet. In recent investigations, it has been confirmed that well-designed soaking and germination stages significantly decreases the phytate and tannin contents in millet grains. In recent studies, it has been resulted that soaking and germination reduced the phytate and tannin substance in millet grains (Elmaki. et al., 2007).
Nelson et al. (2013) have reported that the antinutrient can both increase and decrease in wheat, barley and rice during the germination. Hemalatha et al. (2007) observed that tannin and phytate levels decreases and the bioavailability of iron increases by 20% in finger millet after the germination. Berghofer and Schoenlechner (2010) investigated that processing can destroy and decrease these anti-nutritional compounds such as tannin and phytate. Becker et al. (1981) evaluated 10 different samples of amaranth and found a range of 80 to 420 mg/100 g of tannins. The dark seeds of amaranth contain more tannins than the light ones (Bressani, 1994). According to Venskutonis and Kraujalis, (2013) a very wide range of tannins found in various Amaranthus cultivars, ranged from 0.4 to 5.2 mg/g. Berghofer and Schonelechner, (2002) demonstrated 0.3 to 0.6 % phytic acids in amaranth grains. Phytic acid serves the plant as a form of phosphorus storage.

Cereals and legumes contain higher amount 1 to 3 % of phytic acid; corn contains 0.9 %, soft wheat 1.1 %, brown rice 0.9 %, barley 1.0% and oats 0.8 % (McKevith, 2004). It was found that processing treatments reduced the concentrations of polyphenols by 19 to 59% and tannins by 22 to 59% (Khandelwal et al., 2010). According to Hejazi and Orsat (2016) the germination factors, duration and temperature effects on the protein, peptide, phytic acid, tannin, and oxalate contents of finger millet. The grains germinated for 24, 36, and 48 h at 22, 26 and 30°C shows that both temperature and duration factors significantly influenced the investigated quantities.

Besides this germination decreases the phytic acid, tannin, and oxalate contents by 45%, 46% and 29 %, respectively. The phytic acid content, significantly lowers after germination of field beans, while tannins increased upon processing reported by D’souza, (2013). Shah et al. (2011) studied phytic acid decreases from an initial average value of 1.88 to 0.33 % with 96 h
after sprouting in mung bean. Makinde and Akinoso, (2013) studied effect of processing treatments on anti-nutritional factors of Nigerian sesame (*Sesamum indicum Linn.*) cultivars.

### 2.6 Application of millet in food products

Millets are gluten-free; they have considerable potential in foods and beverages that can be suitable for people which suffers from celiac disease (Taylor and Emmambux, 2008). The extracts of phenolic acids and other bioactive components of millets have to be used as natural alternatives in food preservation and for therapeutic purposes (Xu *et al*., 2011). Lestienne *et al*., (2005) reported that processing and converting millet for use in traditional meals is common in many developing countries in Africa and Asia. In many African countries, millet is often the main component of many meals and is essentially consumed as steam-cooked products (‘*couscous’*), thick porridges (‘*To’*), and thin porridges (‘*Ogi’*) that can be used as a complementary food for infants and young children. Mamiro *et al*. (2001) studied that finger millet and kidney beans (*Phaseolus vulgaris*) were processed by soaking, germination, autoclaving, and fermentation for incorporation into a complementary food for children. The results show that various processing methods, especially germination, increases mineral extractability. Addition of vitamin-C and mango could be used to enhance mineral extractabilities, thereby helping to alleviate micronutrient deficiencies in populations subsisting on these foods.

Almeida-Dominguez *et al*. (1993) reported that porridges prepared from germinated millet and cowpea has high nutritional quality with acceptable properties of weaning foods (an intermediate consistency, smooth texture, and pleasant colour and flavour). The incorporation of germinated millet flour blend is also found to improve the quality of composite flour containing kodo and barnyard millet flour, whole wheat flour and defatted soy flour in terms of increasing
nutrient density, thinner gruel by lowering viscosity, and an increase in the level of syneresis that may improve the resistant starch content on storage (Vijayakumar and Mohankumar, 2009). Adebayo et al. (2010) studied that Kunu is a very nutritious beverage that can supply most of the nutrient requirements by the body. It has analyzed that kunu from millet gives the highest nourishment to the body. It has more nutritive value and is a good source of energy because of the high amount of protein, normal total solids, moderate pH, and acidity. Millet does have a high amount of calcium that helps in healthy bone strength and strong teeth.

Shukla and Srivastava (2011) develops noodle for diabetic patients by using finger millet (30%) and refined wheat flour (50%). Based on the sensory evaluation, 30% finger millet incorporated noodles were selected and evaluated for glycemic response as compared to a control. The results indicated that glycemic index of 30% finger-millet-incorporated noodles was significantly lower than control noodles. Ali et al. (2010) studied that fortification of grain foods was found to be an effective strategy that can be used to overcome nutrient deficiencies. Micronutrient deficiencies, especially of vitamin-A, iron, iodine, and zinc, are widely prevalent in both developing as well as some developed countries. Iron deficiency is a major public health problem in developing countries, it affects up to 50% of infants, children, and women of child-bearing age in poorer populations of Africa, Asia, and Latin America (Ge et al., 2011). It has been established that fortification of millet flours is a technique to enrich them with micronutrients, to improve their nutritional quality. Heat processing of finger millet flour improved the bioaccessibility of iron from both unfortified and fortified flour (Tripathi et al., 2010).

Millets could be used in the preparation of ready-to-eat food products such as in the formulation of whole grain puffed or flaked ready-to eat breakfast cereal with a wide range of
acceptable flavor, color, texture and palatability. It could also be used in the preparation of noodles (Ge et al., 2011). Mallasy et al. (2010) investigated that supplementation of millet flour with soybean protein decreased in vitro protein digestibility with an increase in the portion of soybean in the blend. It has been reported that essential amino acids of millet flour can be enriched on supplementation with soybean protein. Supplementation significantly increased lysine 1.5 to 2.4 fold. Kodo millet flour is used to prepare Kheer, Instant pittu and Pasta (Devi et al., 2014). Singh et al. (2012) reported that for the preparation of breads, millet-based composite flours were optimized. Barnyard millet plus wheat composite flour was formulated and prepared by mixing 61.8 g/100 g barnyard millet, 31.4 g/100 g wheat, and 6.8 g/100 g gluten. The results of sensory analysis show that the acceptability of bread samples prepared from composite flours was almost equal to that of the wheat bread.

The quality of composite flour improves by the incorporation of millet flour blend containing kodo and barnyard millet flour, whole wheat flour and defatted soy flour in terms of increasing nutrient density, thinner gruel by lowered viscosity, and an increase in the level of syneresis that may improve the resistant starch content on storage (Vijayakumar and Mohankumar, 2009). Supplementation of millet grains with natural food products can be a cost effective approach to increase the nutritional value of a diverse range of food materials and could be an alternative to the fortification of foods with chemical synthetic nutrients. The lack of gluten in millet grains could serve as an alternate to wheat for the preparation of bakery products that would be beneficial to patients suffering from celiac diseases.

2.7 Millet based food products and their storage stability

Among the baked product like bread and cakes, cookies have low moisture content which ensures that they are free from microbial spoilage and confer a long shelf life on the product and
long shelf life of cookies makes large scale production and distribution possible (Lean and Mohamed, 1999) and availability almost everywhere at any time (Popov-Raljic 2013). Good eating quality makes them attractive for fortification and other nutritional improvement. Cookies belong to the group of food products that are very popular in daily diet of almost all profiles of consumers (Popov-Raljic, et al., 2007), having not only the nutritive purpose but influencing also on emotional status of consumers with the effects even on the positive mood enhancement.

Cookies are available in different unit packages in various flavours, shapes, sizes and with excellent organoleptic characteristics. The cookies become popular even in traditional food cultures of India due to their excellent shelf life at ambient conditions, simplicity and ease of handling during use and transport and availability at affordable prices for the diverse consumers. The cookies if modified suitably are probably the best vehicles to carry the nutrients to meet the nutritional demand of common consumers (Noorfarahzilah et al., 2014). Results revealed that composite cookies had higher protein, fiber and ash content compared to control cookies.

Similarly, Okpala et al. (2013) reported that 100 % germinated pigeon pea flour (GPF) shows highest protein value. Increase in levels of GPF to the flour blends result an increase in protein content of blends. Cookies made with 100 % fermented sorghum flour (FSF) shows highest ash content of 2.73 %, while cookies made with 100 % GPF showed least ash content. Studies of Chauhan et al. (2015) reported that germination decreased fat content from 6.68 g to 4.7 g/100 g and carbohydrate 62.41 to 60.70 g/100g. While an increase in protein content was noticed from 15.05 to 16.5 g/100 g, total dietary fiber content from 9.52 to 12.9 g/100g and antioxidant activity from 10.23 to 14.71 g/100 g respectively. Similar studies of Baljeet et al. (2014) shows that with increase in concentration of carrot pomace powder (CPP) and germinated
chickpea flour (GCF), there was an increase in protein, ash and crude fiber contents. The biscuits supplemented with 10% CPP and GCF showed highest crude fiber content 3.2%.

Agrahar-Murugkar et al. (2015) reported that nutrient content of CF (composite flour) biscuits (sprouted and unsprouted) were significantly ($p \leq 0.05$) higher than control biscuits made from refined-flour. Studies of Agrahar-Murugkar et al. (2015) also indicated that composite flour (CF) biscuits had significantly ($p \leq 0.05$) lower spread ratio compared to control. Sensory evaluation showed that CF biscuits especially with sprouted flour had higher acceptability and were superior to control. Similarly Okpala et al. (2013) depicted that cookies made with 100 fermented sorghum (FSF) shows least spread ratio of 14.97 than control cookies made from wheat which showed spread ration of 14.97. Chauhan et al. (2015) also reported highest spread ratio in raw amaranth flour cookies and germinated amaranth flour cookies required minimum snap force and exhibited highest antioxidant activity 21.43 g/100g and total dietary fiber 13.97 g/100g compared to raw amaranth and wheat flour based cookies. Similarly, Chung et al. (2014) reported that cookies containing rice flours required significantly less force to compress than did the wheat flour cookie, and this softening effect was increased as the level of rice flour substitution increased. Cookies made with the germinated brown rice (GBR) flour displayed inferior physical characteristics compared to those with wheat flour, but the cookies containing the treated GBR flour shows improved physical properties with lower moisture content and higher spread factor than those containing untreated GBR flour. Overall results showed that cookies with acceptable quality and improved nutrition could be prepared by partial or complete replacement of wheat flour with heat-moisture treated GBR flour. Result of Uchegbu, (2016) also indicated that antioxidant activities in germinated pigeon pea flour were much more than ungerminated pigeon pea flour. The products also had acceptable sensory quality.
According to Baixauli et al. (2008) storage stability or the shelf-life of baked products could be defined as maintenance of the sensory and physical characteristics associated with freshness. In general, cookies have the property of bending after baking unlike biscuits that break. This fact could depend on the cookies have higher water activity and moisture content than biscuits’ (Dhankhar, 2013). There are different types of product changes that can limit the shelf life of food. Essentially, the shelf-life of a food, i.e. the period it will retain an acceptable level of eating quality from a safety and organoleptic point of view, depends on four main factors, namely formulation, processing, packaging, and storage conditions (Fig. 2.1) (Galic et al., 2009).

![Diagram of Factors Influencing Shelf Life](image)

**Figure 2.2 Factors influencing shelf life of food**

Cookies are well-known for their long shelf-life since they are characterized by lower water activity (aw) values than those that permit the growth of microorganisms (aw > 0.6) (Chieh, 2006). Though, they possess a high amount of vegetable fat 20 to 30% on flour weight basis), which makes them susceptible to oxidative changes (Zielinski et al., 2012). Development
of high protein and sugar-free cookies fortified with pea (*Pisum sativum* L.) flour, soya bean (*Glycine max* L.) flour and oat (*Avena sativa* L.) flakes was studied by Amin et al. (2016). The parameters analyzed were storage stability, microbiological analysis and sensory evaluation. Results indicated that cookies were stable both in terms of peroxide and acid values during 2 months of storage period as both the values were within the permissible limits prescribed by Bureau of Indian Standards (BIS). Microbial analysis shows that there was not any microbial growth indicating the products are safe. Sensory evaluation of cookies shows that with regard to color, taste, flavor and texture, cookies with 5 to 10 % pea flour and soya bean flour scored highest. Similarly, Romeo et al., (2010) studied shelf-life of almond pastry cookies with different types of packaging and levels of temperature. Parameters evaluated were texture, water activity and colour. The best results were obtained with aluminum foil (ALL) packaging and modified atmosphere (MAP) condition, and above all in all the trials a temperature of 30 °C reduced the crust hardness.

Studies of Gupta et al. (2011) depicted that cookies were microbiologically safe up to 6 months of storage in different packaging materials under varying temperature conditions. Banusha and Vasantharuba (2014) also reported the bacterial count of 30% of malted flour blend incorporated biscuit upto $3.6 \times 10^2$ CFU/g after 2 months of storage and this is well below the safe level of $1 \times 10^4$ CFU/g. There was no yeast and mold growth observed during two months of storage. Results of Nagi et al. (2012) revealed that acceptability of cereal bran incorporated biscuits was affected with progressive storage; however the product remains in high acceptability range up to 3 months. Free fatty acid content was within permissible limits and microbial count was far below the permissible limits up to 3 months of storage of product in HDPE and laminate at room temperature. Storage stability of cookies developed by substituting wheat flour (*Maida*)
with defatted soy flour (DSF) and cane sugar with stevia leaves powder (SLP) was investigated by Peter et al. (2012). The cookies were then packed in LDPE, HDPE and PP films and stored for a period of 90 days under ambient conditions to assess the quality of the cookies based on sensory and storage parameters. Results indicate that cookies stored in HDPE package have good sensory acceptability.

Among the bakery products, the highest consumption patterns are found bread and cakes. The parameters that attributes to the quality characteristics of cakes include ingredients used, formulation techniques and baking conditions. Quality parameters that are considered better for cakes quality include high volume with appearance along with a homogenous fine structured crumb. The baking of cakes includes three main stages where the first stage involves expansion of batter with loss of moisture, the moisture loss continues in the second stage along with increase in volume of the cake due to releasing of entrapped air in batter and the third stage results in the formation of final products set structured by the denatured protein and gelatinized starch network formation with fine textured crumb (Megahey et al., 2005). The air incorporated in the batter during mixing are the origin of the air pockets which are stabilized by starch gelatinization and protein denaturation upon baking of the cake. The number of air pockets determines the texture of the cake, the higher number of air pockets resulted in more porous structure with higher volume of the cake (Parra et al., 2017).

The quality shelf life of cake is quite short due to the staling process and staling refers to changes that take place after baking other than spoilage by microorganisms. Staling of cake is complex and not fully understood. During storage the most pronounced changes are related to losses in moisture content and hardening of the crumb in cake (Maarel et al., 2002), though flavour and aroma deteriorate as well (Eliasson et al, 1993). The progressive loss of moisture at
room temperature occurs due to diffusion from the crumb to the crust (Stear, 1990). It is believed that cake staling is closely associated with starch retrogradation (recrystallization), involving the primary components of starch, namely amylose and amylopectin. The progressive loss of moisture at room temperature occurs due to diffusion from the crumb to the crust (Stear, 1990). Some of the changes that occur during staling can be reversed by reheating to temperatures of 50–70°C (Eliasson, 1993). Previous research by Guarda et al. (2004) also showed that hydrocolloids can reduce the rate of crumb dehydration in bread during storage. Mohebbi et al. (2017) observed that breads made from β-glucan had higher moisture content than their control after five days of storage. Firmness measurements of the crumb may be used as a method to determine the degree of staling in cake, though moisture content can be another measure since moisture loss is also thought to reduce quality (Stear 1990, Mohamed et al., 2008; Kalinga and Mishra, 2009).

Cake manufacturers face a major problem of lipid oxidation which limits the shelf life of their products (Lean and Mohamed, 1999). Bakery products such as cakes particularly those with high lipid content tend to become rancid after prolonged storage owing to the oxidation of polyunsaturated fatty acids (Smith et al., 2004). Foods containing higher content of polyunsaturated fatty acids are more prone to oxidation (Van Aardt et al., 2004). The FFA content increased from 0.19 to 0.75 % of oleic acid. Increase in FFA results is attributes due to the enzymatic hydrolysis of esterified lipids (Hwang and Regenstein, 1993). Hafez (2012) studied that the peroxide value and thiobarbituric acid in cakes stored at room temperatures for 28 days of storage period under room temperature decreases with increasing marjoram levels in cake. Wagdy and Taha (2012) reported that free acid value and peroxide value of the control butter cake was fortified with jojoba hull at zero time were 0.71%, and 2.7 meq O₂/ Kg., after
three weeks of storage changed to 3.88% and 15.37 meq O₂/kg, respectively. During the initial storage period, peroxide value (PV) of fish cake was found to be 6.12 meqO₂/kg of fat and it increased to 9.97 meqO₂/kg of fat at the end of the 5th day of storage and subsequently decreased to 7.86 meqO₂/kg of fat, at the end of the 15 days of storage period. The decrease of the PV at the end of the storage may be owing to decomposition of hydro peroxides into secondary oxidation products and similar trend was also observed by the Yerlikaya et al. (2005) during the refrigerated studies of fish patties from anchovy.

Microbial spoilage is the major problem causing deterioration of products (Hudson et al., 2007). With abundant nutrients, water activity of around 0.92, and almost neutral pH of 6.7, rice cake can be considered a good medium for microbial growth. For ready-to-eat foods the maximum permissible level of total aerobic colony count given by Fylde Borough Council extracted from manual of PHLSG (PHLSG, 2008) is 10⁴ to less than 10⁶ cfu/g. and international commission on microbiological specification for foods (ICMSF, 2010), also asserted the standard total count below 10⁴ cfu/g, is still considered safe for human consumption. Inanli et al. (2013) reported that fish cake prepared from anchovy also shows an increase in the psychrophilic bacteria count from 1.0×10¹ to 3.60×10⁴ cfu/g of sample during the entire refrigerated storage up to 12 days.

Millets are important staple food across the globe in many regions and are known for health benefits. Most of the health benefits are attributed to its antioxidant properties, dietary fiber contents and polyphenols. Nutritionally, its importance is well recognised because of its high content of minerals, protein, and vitamin. Millets are naturally gluten free source and have a great scope in formulations for celiac disease. The extracts of phenolic acids and
other bioactive components of millets have to be used as natural alternatives in food preservation and for therapeutic purposes.

Therefore, the goal of the present research is to investigate the effect of different processing method (soaking, germination and malting) on the bioactive compounds of foxtail millet, kodo millet and barnyard millet. The research will also be carried out for the utilization of germinated millets in the development of healthy food, followed by quality evaluation and storage studies.