Chapter 16
Effect of Germination Processing on Bioactive Compounds of Cereals and Legumes

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16.1 Introduction

The present global prevalence of lifestyle associated maladies is irrefutable (Essa and El Shemy 2015), with experimental and epidemiological indications devastatingly showing that poor, unhygienic nutrition and lifestyle of most of the people are main trends added to the etiology of long lasting and non-transferable maladies for example over weight, many type of cancers related to diet, diabetes and heart disease (Chaput et al. 2011). Due to upper mentioned maladies nutritionist and diet planners recommend healthy diet which improves health of consumers and reduces risk of diet related disorders (National Health and Medical Research Council [NHMRC] 2013). In current life style people consume food containing high number

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of calories rather than nutrition so causes over weightiness, also modified the normal signaling and homeostasis of body and creates wide range of physiological defects (Fernández-Sánchez et al. 2011). Bunch of maladies produced due to poor diet are diabetes, hypertension, several types of inflammation, dyslipidemia along with considerable risk of heart diseases (Cheung and Li 2012).

Various health conditions could be produced by using different diets, so functional and nutritional components of food are fundamental in controlling and improving maladies produced due to lifestyle (Cencic and Chingwaru 2010). Therefore, consumption of proper and balanced diet along with functional components impacts health most importantly. Now a day's industry of functional foods growing exponentially throughout the world as functional components has many benefits having various physiological effects which proved to be helpful in improving health (Abuajah et al. 2015). Grains (cereals, pulses and legumes) commonly used worldwide are staple foods globally containing many bioactive and functional components affecting health positively and protect consumers from adverse health defects produced to present lifestyle thus received major attention of researchers (Fardet 2010). They are good source of vitamins, minerals, fibre and notable quantities of bioactive compounds including phenolics, carotenoids, lignans, starch, sterols, and phytates. These compounds either have additive and synergistic effects because of having role as free radical scavengers, as cofactors with antioxidant enzymes, or as indirectly performing as antioxidants providing positive effects on human health by reducing the hazards of chronic diseases other than the basic nutrition (Topping 2007).

Among many findings, the application of controlled germination process of seeds is a valuable technique in improving the nutritional contents of these grains which are excessively used in the world. This technique proves to be practical in enhancing the nutritional value of these grains especially in the countries where diet related maladies are major issue due to lifestyle.

16.2 Grains (Cereals and Legumes)

Cereals and legumes rich source of minerals and vitamins (as a whole) are excessively used especially in Asian region. Most of the grains are crushed and converted into flour prior to use while other grains like rice and legumes are used as a whole (Oghbaei and Prakash 2016). If we speak about, these are edible portion derived from caryopsis of cereal grasses, belong to Poaceae (Gramineae) family including many other crops which are grain producing for example barley, rye, wheat, triticale, rice, oats, sorghum and maize (Davidson et al. 2012), along with these some minor grain crops are also included in this family like millet. By description whole grains contain equal amounts of nutrients and grain components (germ, bran, endosperm) and available in various forms such as crushed, cracked, rolled, flaked, cooked or extruded grains (US Food and Drug Administration [USFDA] 2011). Many cereals which do not contain true seeds are considered as whole grain, e.g. amaranth, quinoa and buckwheat, but few grain containing crops like oilseeds and legumes are not included in whole grains (Whole Grains Council n.d.). Furthermore, few grains which were sprouted or malted are also included in whole grains but condition is, they should contain parts like bran, germ, and endosperm and retain their nutritional value (American Association of Cereal Chemists [AACC] 2008).

Cereals are major part of diet of many people throughout the world. Widely growing cops are wheat, maize, rice, sorghum and barley. All these crops belong to a same family called Poaceae, and contain monocotyledon in their grain. Cereals are processed and converted into variety of products and gives energy, dietary fiber, minerals, vitamins and proteins (Sarwar et al. 2013). Besides these cereals are also rich source of many bioactive and functional compounds e.g. tocopherols (antioxidant), phytosterols, phenolic acids, lignans and folates (Van Hung 2016). Consumption of whole grain cereals or their products resulted in many positive effects on health which includes reduction in risk of certain cancers, reduction in level of blood cholesterol and risk of heart related maladies (Sarwar et al. 2013). Several research studies have been conducted using cereals alone or their products as an ingredient for their functional foods (Das et al. 2016).

In developing agricultural countries legume crops also play key role due to their nutritional value for both the producers and consumers. Another form of seeds which are enclosed in pods belongs to legume family defined as "bean" are consumable and nutritious. Legumes also called pulses are grown in various countries. They include wide range of varieties like alfalfa, clover, lupins, green beans, peas, peanuts, dry beans, broad beans, dry peas, kidney beans, fava beans, black beans, soy beans, chickpeas, and lentils etc. In many regions of the world legumes used along with cereals proved to be complementary diet as these are rich source of proteins and minerals, therefore perform a key role in human diet (Bouchenak and Lamri-Senhadji 2013). Dicotyledons contain as many as 700 genera and about 20,000 species among these legumes belongs to family Leguminosae (Petchiammal and Hopper 2014). Among flowering plants on the basis of population Leguminosae is a third most important family (del Socorro López-Cortez et al. 2016).

Plant of legumes recognized by its seeds which are edible and born in pods (Megat Rusydi et al. 2011). Among legumes researchers give their major attention to dry seed besides these dry edible seeds, legumes are also used in the form of salad as these are green in color for example fresh pods, leaves and seedlings (Mitchell et al. 2009). These foods contain many essential nutrients which proved valuable to human health as they contain antioxidant, and are recognized as safe (GRAS) to use for human. Free radicals which are usually produced in biomolecules like DNA, lipids, and other proteins, their oxidative effect is masked by protein of these legumes (Rochfort and Panozzo 2007).

16.3 Biological Activities of Grains

As the grains are used worldwide and are staple foods of many countries therefore their use has the potential of impacting health outcomes. Major portion of grains is carbohydrates which are useful source of energy, it also contains other valuable dietary components e.g. phytochemicals (Okarter and Liu 2010), therefore mixing of these grains into foods either whole grain or in the form of other products gives vital nutritional differences. If we compare nutritional profile of whole grains and milled grains, major difference noticed in dietary fiber, minerals, vitamins and other bioactive compounds e.g. polyphenol which are antioxidants (Jonnalagadda et al. 2011). Various varieties of whole grains contain different nutritional composition and therefore contains different bioactive compounds and activities, so produced various biological effects in body (Donkor et al. 2012). Number of bioactive compounds present in grains along with other components are characterized with different physiological responses (Björck et al. 2012; Fardet 2010). In past many researchers had reviewed the functional characteristics of whole grains, but still they are not clarified completely (Björck et al. 2012). From literature, it is concluded that dietary fiber (important part of whole grains) provides number of health benefits, but data from research shows that all whole grains are not reliable source of dietary fibers e.g. brown rice contain 4.6 g/100 g while dark rye flour contain 23.8 g/100 g (US Food and Drug Administration [USFDA] 2012).

Many explanations regarding mechanism of whole grains activity were discussed e.g. structure of starch, solubility properties and viscosity produced by fiber, along with size of particles (Jonnalagadda et al. 2011), structure of food and gelatinization (Björck et al. 2012). Soluble fiber present in whole grains may help to reduce level of cholesterol in blood by production of bile while production of cholesterol may also be inhibited by shot chain fatty acids which produced after fermentation of grain polysaccharide. Risk of diabetes could be reduced by many minerals (Mg, Se) and antioxidant (Vitamin E) present in whole grains (Jonnalagadda et al. 2011). Oxidative stress and inflammation may also be reduced by polyphenolic antioxidants (Mateo Anson et al. 2010).

Importantly, it was observed that superior health effects were exerted through consumption of whole grains in comparison to milled grains in diet, this effect proved through epidemiological studies (Masters et al. 2010). Basal metabolic rate, LDL and total cholesterol improved by consuming whole grains (Newby et al. 2007). Numerous studies had been conducted related dietary intake of whole grains which associated with reduced risk of various disease but recommended consumption of whole grains is not met in many contraries including Canada, USA and Australia. In developed country like USA recommendation to consume whole grains were 500% more than present consumption while just 20% increment observed in consumption, which were not up-to the mar (Whole Grains Council 2009).

Similar conditions also observed in Australia where less than 1/3rd of whole grain were consumed, which are far less than recommendation (Go Grains Health and Nutrition [GGHN] 2010), In Canadian community, consumption of whole grains was also less than recommendation e.g. data collected in 2004 through a Canadian Health Community showed that between 21% and 66% of Canadian citizens aged 19 years or older were not taking their minimum intake of whole grains (Garriguet 2007). Many reasons discussed regarding reduction in consumption of whole grains, among of these lack of understanding, consumption of refined food and availability of various products have been suggested as obstacles for less whole-grain utilization (Go Grains Health and Nutrition [GGHN] 2010). Besides these barriers many anti-nutritional factors which inherent the grains may also impair the availability of many minerals e.g. iron and zinc such as phytic acid which may inhibit absorption of these mineral (Gupta et al. 2015). A novel grain processing method was recognized by "The European HEALTHIGRAIN" which will improve the palatability and nutritional profile of whole grains and ultimately positively affect the health of consumer (Delcour et al. 2012). Epidemiological and interventional studies mainly focused to non-germinated (non-sprouted) whole-grain and so, creating the gap in understanding many wider effects of whole grains on health.

Additionally, legumes are also rich source of phytochemicals (phytosterols, natural anti-oxidants), polyphenols and other bioactive carbohydrates. Therefore, legumes have increasingly used in diet to treat and prevention of number of dieses in developed countries (Amarowicz and Pegg 2008). Through numerous studies it was proved that correlation exist between consumption of legumes and reduction in risk of several disease e.g. cancer, heart related disease, overweightness and diabetes (Boudjou et al. 2013). As legumes are reliable source of phenolic compounds therefore, antioxidant ability (Heimler et al. 2005) and the anti-cancer (Cardador-Martínez et al. 2002), related to apoptosis (Aparicio-Fernández et al. 2008), and antiproliferative effects are inserted by legumes (Segev et al. 2010).

Many anti-cancer compounds e.g. flavonoids, phenolic acids, lignans, and tannins, which are richly present in legumes (seed coat). Such ant-oxidant (phenolic contents) found in legumes proves to be helpful against many chronic diseases (Aberoumand and Deokule 2008). Legumes are usually as a seed but many products could also be produced from legumes such as dhal, snack foods, flour etc. which could be used for further food preparation (Villegas et al. 2008) or also as germinated grains. It is universally accepted that simple and less costly conventional processing methods are effective techniques to bring useful changes in composition of legume seeds. Among these conventional techniques soaking, cooking, fermentation and germination may be used to enhance the quality of legumes and their nutritional value due to the removal of few anti-nutritional factors (Singh et al. 2014).

16.4 Germination Processing

Germination is a natural processing technique used for biological activation of grains to improve their nutritional and functional properties (Hefni and Witthöft 2011). Sometimes seeds germination in controlled environment is achieved to get desire benefits in which seeds are grown thus sprouts formed during germination process. The environmental conditions required for germination are optimal humidity level, accessibility of oxygen (aerobic respiration), acceptable temperature for proper time duration to stimulate metabolic processes (Sangronis and Machado 2007). These environmental factors could be controlled like moisture, temperature and humidity in controlled environment in the laboratory or alternatively seed could also have germinated in field, process called preharvest sprouting (PHS). In both condition, basic purpose is sprouting which resulted in higher amounts of essential nutrients like soluble dietary fiber, vitamins, minerals, antioxidants and phytochemicals which proved beneficial for consumers (Van Hung et al. 2011). It's necessary to carry out germination for several days to increase nutritional value of grains (Rakcejeva et al. 2014).

Additionally, sprouts are also rich source of health improving vital nutrients like glucosinolates, phenolics and selenium-containing components in various legumes for example in the Brassica plants and isoflavones in the soybean plant. It is very important to note that the sprouts which produced during germination are consumed as such so gives high amount of nutrients. Addition to nutrients sprouts are also rich source of various phytochemicals, various minerals and vitamins, enzymes and essential amino acids which exert heath promising effects on humans (Gan et al. 2017). Recently researcher gave more attention to biological value of the nutritional sprouts (Marton et al. 2010). Many anti-nutritional compounds such as trypsin inhibitor, phytic acid, pentosan, tannin etc. reduced in considerable amount, and on the other hand it also observed many compounds which proved to be health improving compounds are increased which showed anti-cancer activity, example of these compounds is glucosinolates. Therefore, sprouting can increase the amount of nutrients which exert positive effect on human health and reduces the risk of many serious diseases (Sangronis and Machado 2007).

Exact nutritional composition of sprouts is not clear as nutritional profile varies with variety of legumes but fundamentally germination modified the nutritional and physicochemical characteristics of seeds to support the growth of baby plant (Noda et al. 2004). As compared to non-germinated (non-sprouted) grains, germinated exert beneficial effect on human health as they contain huge amount of beneficial nutrients and antinutritional factors are also reduced during germination process. For germination grains are soaked in water for the short time duration which facilitate the germination process resultantly improve the technological and nutritional properties of grains. Alongside the modification in nutritional value germination also changes the appearance, taste, and flavor of the grains (Kaukovirta-Norja et al. 2004). In germination process kernels are usually soaked in water and after that allowed to germinate in controlled environment. Therefore, sprouting conditions and water contents exert significant impact on metabolic process of grains. And this metabolic process occurs in embryo results in the formation and/or release of metabolic compounds which carry health maintaining effects (Hübner and Arendt 2013).

16.5 Mechanism of Germination

Germination processing can be conducted in different simple steps like sterilization, steeping and sprouting. Germination can be activated in controlled environment having optimum range of temperature, light and moisture factors. Before soaking the seeds in water, sterilization is carried out to reduce the microbial load. Many sterilization solutions available but solution of sodium hypochlorite (NaClO) with various concentration proved to be promising and is commonly used to reduce to microbial load before seed germination (0.07% NaClO solution) (Limón et al. 2014). By using above mentioned solution usually sterilization is carried out at room temperature for 15–30-min duration in the ratio of 1:5 or 1:6 with 85 (g) seed weight/solution volume. Beside sodium hypochlorite pure ethanol or ethanol 70% solution and 0.2% formaldehyde solution also successfully used in literature for 3-min time duration (Nour et al. 2015; Pająk et al. 2014). Results of few research shows effect of sterilization process on seed germination. While in few research sterilization, process is omitted (Guo et al. 2012; Guajardo-Flores et al. 2013), researcher claim that sterilization process can cause potential hazardous effects and create seed food safety risks. So, sterilization of seeds is not compulsory before seed germination, it depends upon many factors including seed condition, water changing frequency during germination process and germination purpose.

Before carrying germination, seed is usually soaked in water to re-hydrate the seed and during this process temperature, time of soaking, ratio of seed weight and water volume should be considered. Soaking the seed at room temperature from few to 24 h and seed weight/volume ratio of 1:1.5 to 1:20 is a frequent practice which carried out before seed germination. The variation in seed soaking depends upon inherent characteristics of seeds for example water absorbing capacity of seeds, seed coat thickness and seed size. After carrying out soaking process seeds could be placed in incubators for proper germination (Gan et al. 2017). The metabolic activity of dry grains boosts rapidly as it is hydrated during soaking. For proper seed germination process several processes should be note such as the light, temperature, humidity, watering and time. Successful germination usually performed in dark and temperature for germination is kept 20 to 30 °C. Proper care is required during germination process, for example watering to should be done every day to provide seed optimum humidity for growth, water should be replaced periodically (twice a day), it will help to eliminate metabolites of sprouted seed and stop the growth of microorganisms (Kandil et al. 2015). To start germination in the grains presence of water is compulsory which initiates extensive biochemical and physiological process which provides support to growing baby plant and this process ceased after elongation of embryonic axis. Emergence of radical round the embryo of the grain is the visible sign that germination had completed (Bewley and Black 1994). Water penetration enhances seed rehydration which stimulates gibberellin synthesis in embryo which leads to gene expression of hydrolytic enzyme (Nelson et al. 2013).

As grains always remain in dormant in unfavorable condition therefore, for proper germination grains are exposed to appropriate environmental condition e.g. proper temperature and moisture which will stimulate the hormones present in grains to initiate the germination process. Optimum moisture contents and temperature will stimulate the debranching and hydrolytic enzymes and some hormones which will liberate nutrients from germ (embryo), endosperm and scutellum from within the seed (Gan et al. 2017). Period for sprouting usually varied seed to seed for example 3–5 days are enough for successful germination of edible beans. Germination process is simple, less costly, environmental friendly and recommended as safe to sprout a seed within less duration of time (Gan et al. 2017).

During germination process, complex biochemical changes occur in seeds like hormones (gibberellin) release from embryo and reached up-to aleuronic layer of seed and stimulate the release of different enzymes (amylases, proteases) into the endosperm, and also lower the activity of enzyme inhibitors, all these activities will act on compounds present in germ and endosperm therefore, convert seed from dormant to active metabolism (Iordan et al. 2013). These enzymes will act on stored carbohydrates and proteins and convert them to smaller molecules which will be used by the baby plant for growth. It is interesting to know that most of these stored compounds are not soluble into water therefore growing embryo unable to use them until they converted to smaller components by enzymes which are soluble (Miransari and Smith 2014).

As the germination process starts in the grains numerous phytochemical and physiological changes took place in which complex nutrient flux occur which includes degradation, remobilization and accumulation. Stored complex compounds e.g. starch, lipids and proteins are catabolized during germination which resultantly produced smaller molecules such as nitrogen and carbon which are used for the proper growth and photosynthesis by the plant (Theodoulou and Eastmond 2012). During germination process lot of nutrients and other bioactive compounds increased (Donkor et al. 2012), and reduced as these compounds consumed by the growing plant (Yang et al. 2001). It was given by Hung et al. (2012) that sprouted waxy wheat had improved and beneficial nutritive profile as compare to non-sprouted, as it contains high amount of dietary fiber, free amino acids and phenolics. In another study, it was reported that after 102 h germination at 20–25 °C grains contain 2 times high amount of α-tocopherol and 2–3 times more minerals (Ozturk et al. 2012). In same study 3.6-time higher amount of folate was found (Koehler et al. 2007). Another research conducted by Yang and co-workers (2001), reported that amount of ascorbic acid and tocopherol and β-carotene were not easy to detect in un-germinated wheat grains, while in the same grains these compounds were present in higher amounts and their amount continuously increasing after lengthening the time of germination and reached up-to optimum value after 7 days of germination. Values of vitamin C were 550 μg/g, and of α-tocopherol were 10.92 μg/g while for β-carotene were 3.1 μg/g. In the same study amounts of ferulic and vanillic acids were also noticeably enhanced, reached optimum level after 7 days of germination which became 932.4 μg/g and 12.9 μg/g, respectively.

Many variables such as variations in grain types, germination conditions along with laboratory technologies may confused the findings. Due to these variable results achieved after germination of same type of whole grain could be changed (decrease or increase) or unchanged. Such as amount of sugar were resulted both increase or decrease when compare with control group (un-germinated) while different results were showed by using different type of rice varieties. (Moongngarm and Saetung 2010). Related results were also found in another study in which antioxidant ability of sprouted rice resulted both increase and decrease, antioxidant activity was measured by using scavenging activity of the rice variety (Imam et al. 2012). Due to above mentioned reasons care should be taken during comparison of the results from other studies. It proved from previous discussion that optimum conditions might be varied with grain type, parameter to be studied and analytical techniques being used for analysis. The degree of the variation detected may change with other parameters like temperature, time, moisture, available oxygen for germination (Koehler et al. 2007), type of whole grain and environmental conditions (Mak et al. 2009).

If talk about life-cycle of the cereals grains it comprised upon two phases which were separated from each other by dormancy i.e. germination and development. During germination seeds play a vital role as a reproductive unit and this unit guarantees survival of all species of plants. There are three distinct parts of the wheat grains namely endosperm, bran layer (sometime called peripheral layer) which comprised of aleurone, nucleus tegument, testa and pericarp, and important part germ which is comprised of embryos and scutellum (Tasleem-Tahir et al. 2011). As the germination process starts complex compound present in grain like carbohydrates, protein and lipids are converted to simple and soluble molecules by the action of various enzymes which are present in the kernel. These enzymes usually stimulate upon germination. Mostly such enzymes present in upper aleurone layer, bran and in germ (Poutanen 1997). For example, amylase enzyme required to break-down complex starch usually present in the pericarp. Another important enzyme required to break-down complex protein (proteases) are present in endosperm, germ and aleurone layer. Lipoxygenase enzyme which break-down fats is dominantly present in embryo, while polyphenol oxidase and peroxidase are dominantly present in bran of seeds (Rani et al. 2011). These enzymes convert complex compounds into simple fractions which initiate the complex physiological and bio-chemical changes in the seeds.

16.6 Bioactive Compounds

A vast number of organic compounds are produced by the plants and these organic compounds didn't take part directly in growth and development of the plants. Conventionally, such compounds are called secondary metabolites and are found in various amounts in taxonomic group of plants. In contrast, secondary metabolites plant also produced primary metabolites such as acyl acids, phytosterols, organic acids and amino acids which are produced by all types of plants and participate essentially in development and growth of the plant (Hussain et al. 2012). Secondary metabolites which are limitedly produced by the plants have complex structure and biosynthetic pathways and conventionally these compounds didn't get much intentions of the researchers as these compounds are taken biologically insignificant (Parsaeimehr et al. 2011).

In contrast to biochemists, pharmaceutical researchers gave much intention to secondary metabolites (organic compounds) and since 1850s they intensively investigated their biochemical properties. Interestingly, investigation on these bioactive compounds was not just on academic level but on industrial level several compounds were developed from these bioactive components, few examples are dyes, polymers, fibers, glues, oils, waxes, flavoring agents, perfumes, and drugs. These compounds have huge biochemical properties due to which researchers focused to develop innovative drug, herbicides, insecticides and antibiotics (Grindberg et al. 2011).

Based on their synthetic pathways, these compounds are divided into three major categories (nitrogen containing compounds, terpenes and phenolics) (Krzyzanowska et al. 2010). Research had been conducted on their mechanism of synthesis including firstly the mevalonic pathway (to synthesize terpenes), secondly shikimic acid pathway or the mevalonic pathway (to synthesize phenolic compounds), thirdly tricarboxilic acid pathway (to synthesize nitrogen containing secondary metabolites). In pharmaceutical industry, vast number of products are produced by using these secondary metabolites (Parsaeimehr et al. 2011).

16.7 Phenolic Compounds

In numerous studies, (Table 16.3) polyphenolic compounds recognized as major antioxidant components of the whole grains (Zieli'nski and Kozłowska 2000). Radical compounds produced from oxidation are stabilized by the phenolic compounds as phenolic compounds are antioxidant in nature. Whole grains contain large amount of antioxidant compounds, few examples of such compounds are vitamins, sterols, and phenolics as well as phytic acid. Above mentioned compounds mostly found in bran or germ of the whole grain (Fardet et al. 2008). All these mentioned bioactive compounds are participated in degree of antioxidant properties and germination process also affect such compounds. Various in vitro studies showed the antioxidant effect of these compounds however, in vivo antioxidant effect is not completely recognized yet (Fardet et al. 2008). It is interesting to know that few bioactive compounds might act as synergists/antagonists, having antioxidant characteristics in-vitro, might not observed of such compounds in-vivo. Polyphenols primarily occur in both soluble or bound forms in plants but edible grains mostly have high ratio of bound phenolics (Agati et al. 2012).

Polyphenolic contents (secondary metabolites) produced by plants contains variety of structurally and functionally diverse compounds which are produced through the shikimate-phenylpropanoids flavonoids pathways (Krzyzanowska et al. 2010). Majority of the phenolic compounds show estrogenic activities, and defined as the compounds having one or more aromatic ring along with hydroxyl substituents, including many functional derivatives like esters, methyl ethers, glycosides, etc. (Parsaeimehr et al. 2011). Polyphenolic compounds could be divided into two categories, firstly soluble phenols e.g. phenolic acids, flavonoids and quinones, secondly insoluble phenols e.g. condensed tanins, lignins and cell wall bounded hydroxycinammic acids (Krzyzanowska et al. 2010).

Many types of compounds are included in polyphenolic compounds like furanocoumarins, lignin, flavonoids, isoflavonoids, and tannins. Another class of phenols flavonoids comprised of large and varied units of polyphenolic compounds found in plants. Among flavonoids vital group of compounds are flavonols comprised of quercetin, kaempferol, and isorhamnetin, other important group is flavones for example apigenin, luteolin, and chrysoeriol (Hounsome et al. 2008). Another class called phenolic acids contains gallic and caffeic acids, vanillic acid, cinnamic acid and coumaric acid (Krzyzanowska et al. 2010). Most commonly phenolic acids and flavonoids are found in sprouted seeds which have recognized as anti-oxidant and could be used as functional compounds (Fu et al. 2011). As germinated (sprouted) are rich source of poylphenolic compounds and other bioactive compounds therefore, these seeds could be the best alternative of fruits and vegetables in our healthy diets.

16.8 Germinated Grains and Polyphenols

Through several studies in cereal grains it was shown that germination process could enhance the amount of solvent-extractable phenolic compounds (Tables 16.1–16.3). Several authors suggested that increment of water soluble polyphenolic compounds during sprouting process can be attributed to the *de novo* synthesis and transformation (Kim et al. 2013; Tang et al. 2014). Negative impact of seed germination also observed, as many studies showed the reduction in polyphenolic content in sprouted seeds. And this contradictory result associated with increment in moisture content during germination process, during which soluble phenols may be wasted (Guo et al. 2012). These opposing results stated are mainly accredited to difference in soaking, temperatures, germination time and methods of drying the germinated grains. Increased phenolic contents and antioxidant activities has been stated through different *in vitro* studies in cereal grains, like wheat (Van Hung et al. 2011), sorghum (Donkor et al. 2012), buckwheat (Alvarez-Jubete et al. 2010), and rice (Imam et al. 2012).

Enhanced polyphenol oxidase activity (PPO) might be the reason of increased polyphenol contents in germinated grains as compared with non-germinated grain (Demeke et al. 2001). The increase in polyphenols during seed geminating could be due to solubilization of condensed tannins during water soaking and its movement to the outer layer during germination. During maize germination phytate contents

Table 16.1 Phenolic compounds in different germinated cereals sprouts

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Maize	Phenolic contents	UV spectrophotometer	Increased	Mihafu et al. (2017)
Barley	Total flavonoid content Total phenolic content Individual polyphenols (gallic acid, vanillic acid, catechin, epicatechin, chlorogenic acid, ferulic acid sinapic acid, myricetin, quercetin, kaempferol) Antioxidant activity	UV spectrophotometer HPLC DPPH & ABTS,	Increased	Aborus et al. (2017)
Soybean	Total flavonoid content Total phenolic content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Xue et al. (2016)
Buckwheat	Individual phenolic compound (qrientin, isoorientin, vitexin, isovitexin, rutin)	HPLC	Increased	Nam et al. (2015)
Wheat	Total phenolic content	UV spectrophotometer	Increased	Van Hung et al. (2015)
Soybean	Total phenolic content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Koo et al. (2015)
Soybean	Antioxidant activity	UV spectrophotometer	Increased	Victoria et al. (2015)
Wheat	Total phenolic content Individual phenolic compound (ferulic acid, isoferulic acid) Antioxidant activity	UV spectrophotometer HPLC FRAP, DPPH, ABTS	Increased	Zilic et al. (2014)
Finger millet	Polyphenols content	UV spectrophotometer HPLC	Increased	Sudha and Usha (2014)
Barley, Oat, Wheat	Polyphenol content Antioxidant activity	UV spectrophotometer FRAP	Increased	Panfil et al. (2014)
Soybean	Total phenolics Antioxidant activity Isoflavone content (daidzein, glycitein, genistein, total aglycones, daidzin, glycitin, genistin)	UV spectrophotometer DPPH HPLC	Increased	Huang et al. (2014)

(continued)

Table 16.1 (continued)

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Rye, Sorghum, Brown rice, Wheat, Oat, Barley, Buckwheat	Total polyphenol content Individual polyphenol content (gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, *p*-coumaric acid, ferulic acid, luteolin) Antioxidant activity	UV spectrophotometer HPLC DPPH	Increased	Donkor et al. (2012)
Rice	Total polyphenol content Antioxidant activity	UV spectrophotometer DPPH, ABTS	Increased	Imam et al. (2012)
Soybean	Total phenolic content Antioxidant activity	UV spectrophotometer FRAP	Decreased (fresh weight basis)	Shohag et al. (2012)
Waxy wheat	Total phenolic compounds Antioxidant activity	UV spectrophotometer DPPH	Increased	Hung et al. (2012)
Wheat	Total polyphenol content Phenolic compounds profile (hydroxybezoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic) Antioxidant activity	UV spectrophotometer UPLC DPPH	Increased	Van Hung et al. (2011)
Rice	Total polyphenol content	UV spectrophotometer	Non-significant change	Moongngarm and Saetung (2010)
Amaranth, Buckwheat, Quinoa, Wheat	Total polyphenol content Individual polyphenol content (protocatechuic acid, protocatechuic acid, vanillic acid derivative, vanillic acid, caffeic acid derivative, syringic acid derivative, caffeic acid, caffeic acid derivative, 3-coumaric acid derivative, ethyl gallate) Antioxidant activity	UV spectrophotometer HPLC DPPH, FRAP	Increased	Alvarez-Jubete et al. (2010)
Rice	Ferulic acid	HPLC	Increased	Banchuen et al. (2009)

Table 16.2 Phenolic compounds in different germinated legume sprouts

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Adzuki bean	Total phenolic content Total flavonoid content Phenolic acids content Antioxidant activity	UV Spectrophotometer DPPH, ABTS	Increased	Złotek et al. (2015)
Mung bean	Total phenolic content Antioxidant activity	UV Spectrophotometer FRAP	Increased Decreased	Tiwari et al. (2017)
Faba bean, White bean, Chickpea, Lentil, Fenugreek seeds	Total phenols Total flavonoids Individual phenolic compounds (ferulic, pyrogallol, protocatechuic, catechin, syringic, epicatechin, vanillic, gallic, caffeic, chlorogenic, ellagic and coumarin) Total antioxidant	UV Spectrophotometer HPLC DPPH	Increased	Salem et al. (2014)
Pigeon pea	Total phenolic content Antioxidant activity	UV Spectrophotometer DPPH	Increased	Uchegbu and Ishiwu (2016)
Mung bean	Total Phenolics Antioxidant activity	UV Spectrophotometer DPPH	Increased	Kim et al. (2012)
Mung bean	Total Phenolics Total flavonoid Individual phenolic compounds (quercetin, myricetin, quercetin-3-O-glucoside) Antioxidant activity	UV Spectrophotometer HPLC-UV Hydro-PSC	Increased	Guo et al. (2012)
Green mung, Arhar, Masur, Chickpea	Total phenolic content Antioxidant activity	Colorimetric method DPPH	Decreased Increased	Singh et al. (2014)
Lentil	Total phenolic compounds Antioxidant activity	UV Spectrophotometer Oven test method	Increased	Gharachorloo et al. (2012)
White bean, Common vetch, Lentil, Chickpea	Total phenolic contents Antioxidant activity	UV Spectrophotometer Oven test method	Increased	Gharachorloo et al. (2013)
Black gram, Desi chickpea, Cowpea, Yellow mustard	Total antioxidant Total phenol content Total flavonoid content	DPPH UV Spectrophotometer	Increased	Khyade and Jagtap (2016)

(continued)

Table 16.2 (continued)

Grain type	Phenolic compound	Analytical method	Remarks/ results	References
Green mung bean, Black mung bean	Total phenol content Individual phenolic compounds (gallic acid, *p*-coumaric acid, catechin, rutin, vitexin and isovitexin) Antioxidant activity	UV Spectrophotometer HPLC ABTS, FRAP	Increased	Gan et al. (2017)

reduced which could be credited to the increased activity of endogenous enzyme phytase, which hydrolyzes phytic acid (Pawar et al. 2006). The level of the phytate breakdown depends upon several factors like grain variety, germination stage, pH, moisture level, temperature, solubility of phytate and the presence of certain inhibitors (Egli et al. 2002). In a study conducted by Liukkonen et al. (2003) rye grains were germinated for 6 days at different temperatures like 5, 10 and 25 °C all treatments exhibited an increment in methanol-extractable phenolic compounds but highest increase was observed in samples germinated at 25 °C. The reason suggested for this increase was that synthesis or activation of a variety of hydrolytic enzymes started in grains through germination which caused different alterations in structure or synthesis of new compounds having high bioactivity and nutritional value (Wang et al. 2014).

In other study Tian et al. (2004) found that during germination for 24 h in brown rice main soluble phenolic compounds including 6-*O*-feruloylsucrose and 6-*O*-sinapoylsucrose were decreased, while the free compounds including ferulic acid and sinapinic acid increased significantly. In soybean and mung bean after germination total phenolics content in seeds and sprouts expressed on a fresh weight basis (Shohag et al. 2012) decreased compared with other seed contents due to dilution effect of phenolics after imbibition and growth, and due to increased water absorption. While in case of dry weight basis the total phenolics content increased with germination time, because moisture content is eliminated (Cevallos-Casals and Cisneros-Zevallos 2010).

Total antioxidant activity has been generally associated with increased antioxidant activity in a variety of grains (Imam et al. 2012). Avenanthramides are phenolic compounds exclusively found in oats. They have been reported to contribute to the fresh taste of oat products. An increase in these compounds by about 20% upon germination was reported (Skoglund et al. 2008). Tannins, phenolic compounds with high molecular weight, have traditionally been nutritionally negative, as they can form insoluble complexes with proteins and can complex minerals. On a positive note, they contribute to the antioxidant activity and the breakdown products also have antioxidant properties. During germination, tannins are partially broken down, as reported for millet (Hemalatha et al. 2007), and sorghum (Dicko et al. 2005).

Table 16.3 Commonly present phenolic acids in cereal grains

Phenolic acids	Predominant	Major	Low amount	References
Cinnamic acid	–	Millet	Wheat, sorghum, oat	Dykes and Rooney (2006, 2007) and Shahidi and Naczk (2004)
Caffeic acid	–	Oat, sorghum, rice, rye, barley, wheat	Maize, rye, millet	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
p-Coumaric acid	–	Maize, wheat, barley, rye, sorghum, millet, rice, oat	–	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Ferulic acid	Maize, wheat, barley, rye, millet, sorghum, rice	Oat	–	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Gallic acid	–	Rice	Sorghum, millet, oat, wheat	Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
p-Hydroxy benzoic acid	Barley	Oat, rice, millet	Wheat, maize, rye, sorghum	Beta et al. (2012), Cai et al. (2011), Dykes and Rooney (2007), and Laokuldilok et al. (2010)
Protocatechuic acid	–	Rice, sorghum	Millet, rye, barley, oat, maize	Beta et al. (2012), Cai et al. (2011), Laokuldilok et al. (2010), and Zhang and Hamaker (2012)
Sinapic acid	–	Rye, rice	Wheat, maize, barley, sorghum, millet, oat	Beta et al. (2012), Cai et al. (2011), and Laokuldilok et al. (2010)
Salicylic acid	–	–	Wheat, barley, sorghum	Dykes and Rooney (2007) and Kim et al. (2006)
Syringic acid	–	Sorghum, wheat, maize	Oat, millet, barley	Dykes and Rooney (2007), Kim et al. (2006), and Zhang and Hamaker (2012)
Vanillic acid	–	Barley, oat, millet, wheat	Sorghum, rye, maize	Beta et al. (2012), Cai et al. (2011), and Zhang and Hamaker (2012)

Among whole seeds plant, legumes have very wide range of antioxidant activity, as it relies upon variety and origin of plant. While if we review the impact of seed germination and technological processing also effect the inherent ant-oxidant activity of the seeds of legumes for example phenolics, tocopherols, vitamin C etc. Currently researchers greatly interested in function and efficiency of anti-oxidants present in food components which increase the attentions to test the anti-oxidant activity of foods. Therefore, some researched had worked on the germination effect upon nutritional profile of legumes (Gharachorloo et al. 2012). Lentil sprout flour could be the extremely good source of natural anti-oxidants as germination modify phenolic compounds therefore, their anti-oxidant activity also improved. Research data showed that germination process improves the quantity and quality of polyphenolic compounds in legumes (López-Amorós et al. 2006).

Sprouting is a natural process improving the nutritional value of legumes due to increasing phenolic contents. According to one study (Tiwari et al. 2017) of mung beans it may be possible that germination caused enzymatic degradation of carbohydrates which in results increased polyphenols production (Perron and Brumaghim 2009). Results of this study showed decreases the antioxidant activity because boiling of sprouted mung bean may cause denaturation of proteins and enzymes having antioxidative properties. According to several other studies researchers shown that during germination high temperature causes decrease in many vitamins, protein contents and secondary metabolites in food grains (Soris et al. 2010). In another study conducted by Salem et al. (2014) showed an increase in total flavonoids, total phenolics and total antioxidant activity. Increase in phenols may be due to solubilization of condensed tannin during soaking process and relocation of phenols to the outer layer during germination as appeared through browning of the germinated seeds (Sokrab et al. 2012).

But further study requires describing the composition of the seed extract for identification and determination the level of biologically active compounds. Further data required to shows the effect of food processing technologies and various physiological process (digestion) on the availability and stability of these functional and bio-active compounds for using lentils for food supplement (Malik and Kapoor 2015). A large no of research had been conducted in past which shows a direct relation with consumption of foods and reduction in risk of many diseases like cardiovascular and cancer (Fernandez-Orozco et al. 2009). As many health benefits are related with anti-oxidants compounds of the foods, therefore pulses are intensively studied for their anti-oxidant activity. Furthermore, data also present on modification of their anti-oxidant components while processing which also create interest in carrying research. Many compounds are present on pulses which shows anti-oxidant activity, major compounds are ascorbic acid and tocopherol, phenolic compounds and reduced glutathione. From many years sprouted seeds had been used in healthy diet as they provide phytochemicals which have ability to reduce the incidence of various diseases (Fernandez-Orozco et al. 2006).

16.9 Future Perspective

All over the world cereal grains and legumes remain an important staple diet making considerable involvement to intake of different nutrients including carbohydrates, proteins, vitamins, minerals, and fiber as well as different bioactive compounds (Gani et al. 2012). Different health benefits provided by cereals grains and legumes are due to these bioactive compounds present in them. These compounds having covalent bounding with indigestible polysaccharides are key source of antioxidants in legumes and cereal grains. Due to interference created by these covalent bonds, most of phenolic compounds are not properly bioavailable to body because they are not available for enzymatic digestion in human gastrointestinal tract. However, their bioavailability could be improved by increasing their availability to enzymes in gastrointestinal tract mainly through decreasing particle size, interruption of structural matrices, and their release from matrices by using appropriate processing techniques (Wang et al. 2014).

Different processing techniques their parameters and grain types affect the bioavailability of phenolic compounds from cereals and legumes. The nutritional profile of grains can be improved through germination leading to products with better nutritional and sensory properties having improved bioavailability in body as compared to raw form grains. Major objective of germination is to activate hydrolytic enzymes from their inactive form in raw seeds (Ayernor and Ocloo 2007). Moreover, as germination has been claimed of improving the nutritional quality of grains through softening their structure and reducing antinutritional factors so have potential for the industrial applications in development of new ingredients, reengineer processes, and products (Tian et al. 2010).

More research work is required for isolation and characterization of these bioactive compounds after germination from sprouts contributing to beneficial health effect. Research is also desired in determination of metabolism, bioavailability and health influence of these compounds in humans because grains contain varied range of compounds having antioxidant potential.

16.10 Conclusion

Seed germination an important economical processing technique which improves nutritive value and profile of bioactive compounds in grains by removing antinutritional factors and enhancing their digestibility. In addition to changing the nutritional level and biochemical activities, germination process through enzymatic actions also produces bioactive compounds and increases antioxidant activity having health-promoting activities. Germinated biologically stimulated grains should be widely recognized and used as functional foods due to their nutritional and health related benefits. The biological stimulation in grains through germination can enhance their functional properties which could be further used to formulate new healthy functional food products.

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