Germination: an alternative source to promote phytonutrients in edible seeds

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Received 17 October 2019; Revised 29 November 2019; Editorial decision 9 December 2019.

Abstract
Consumption of less phytonutrient foods has shown to cause different chronic diseases, despite over 50,000 edible plant breed available in various countries around the globe. These edible plants consist of seeds that can be consumed which possessed high health benefits. Moreover, nutritive values such as phytochemicals of edible seeds increased after germination. Therefore, germination has been reported to enhance various bioactive compounds such as γ-amino butyric acid, polyphenols, and vitamins which lead to greater bioactivity such as anti-diabetic, anti-bacteria, and anti-cancer effects when these seeds are consumed. Consequently, germination can be regarded as a cheap and effective way to enhance the nutritional value of edible seeds.

Key words: edible seeds; water; bioactive compounds; germination; phytonutrients.

Introduction
Germination is a process that involves imbibition of water by a quiescent dry seed which leads to the development and growth of the seed (Nonogaki et al., 2010). In addition, germination is highlighted as a principal bio-processing method in the field of food science and nutrition because it brings about increase in bioactive compounds. According to Bewley et al. (2012), germination incorporates those events which involves the uptake of water by the quiescent dry seeds and terminate with the elongation of the embryonic axis. Watanabe et al. (2004) stated germination as a biological process initiated once dry seeds absorb water which leads to activation of enzymes with a set physical condition desirable for the sprouting of the seeds. Dogra et al. (2013) reported that germination activates seeds from its dormancy which restores the seeds metabolic activities and leads to biochemical, nutritional, and sensorial changes of the seeds. According to Pimentel et al. (2000), over 50,000 edible plants species in the world have been reported to be available for consumption. It was also reported that edible seeds contain various phytochemicals that provide anti-oxidant, anti-diabetic, and anti-cancer effects.

Many studies have shown that germination can further increase nutritive and bioactive compounds in edible seeds (Saleh et al., 2013; Huang et al., 2014; Fouad and Rehab, 2015). During germination, degradation of macro-nutrients occurs, for example, carbohydrate, protein, and fatty acid are further broken down into glucose, fructose, free amino acid, and organic acids (Shi et al., 2010). Therefore, it is necessary to discuss the activities involved in effecting germination and the resulting changes in bioactive compounds obtained as shown in Figure 1.

Steps to Germination
To facilitate a germination process, there are various steps to carry out as highlighted below:
Decontamination
Although intact seeds are usually germ-free, various steps during harvesting such as hand picking, transportation, and drying make seedcoat to be contaminated with dust and micro-organism. Generally, the seeds are dried to a moisture content of around 8% before storage; thus, the microorganism stained on or even in the seeds could not function. However, when the seeds are soaked in water, the environment becomes easier for the microorganisms to grow and invade the intact seeds leading to spoilage if no decontamination process is applied. Therefore, chemicals including sodium hypochlorite (NaClO) are used to kill the microbes. According to Pajak et al. (2014) and Wu et al. (2012) who reported that NaClO concentration for decontaminating seeds legumes and cereals seeds may vary, but it is usually in small quantity of about 0.5–5% (Selcuk et al., 2008; Bhat et al., 2010). However, excessive decontaminant can be toxic to the seeds and affect the human health when toxic seeds are consumed.

Soaking
The essence of soaking seeds in water is to rehydrate the seeds before germination occur. Parameters such as seed weight/water volume ratio, time, and soaking temperature are very important and should be considered before soaking the seed. In addition, frequent change of water should be implemented, for instance, twice a day, for removal of metabolites in germinated seeds and retard the growth of micro-organisms. Excessive soaking time of rice leads to microbial enrichment and fermentation (Ray et al., 2016). In contrast, insufficient soaking does not support the augmentation of phytochemical content (Chaiyasut et al., 2017).

Sprouting
There are several factors to consider before a seed can sprout. These factors include light, temperature, humidity, water, oxygen, and temperature. Chaiyasut et al. (2017) reported that germination is supported by sufficient oxygen to allow the seeds respires, suitable temperature to allow various metabolic processes during germination. Moongngarm and Saetung (2010) reported that rice seed can germinate anaerobically through a rapid elongation of coleoptile but the radicle could not protrude well under this condition. However, once it is switched to aerobic condition, the radicle can continue to elongate, which suggested that oxygen availability is a determinant factor for true germination (Menegus et al., 1991). The soaking time and temperature depend on the seeds and cultivars; however, several studies have reported that 25–30°C is suitable temperature for germination (Bandara et al., 1991; Capanzana and Buckle, 1997). Germination in the presence of darkness results in stress condition which makes glutamate decarboxylase convert glutamic acid to γ-aminobutyric acid (GABA) (Bai et al., 2009). In addition, during sprouting seeds are needed to be sprinkled with water on a daily basis to keep the relative humidity high in order to support their growth.

Influence of Germination on Bioactive Compounds in Edible Seeds
Studies have shown that germination brings about accumulation of different bioactive compounds (Saleh et al., 2013; Gan et al., 2017). These bioactive compounds include GABA, gamma oryzanol, ferulic acid, polyphenols, vitamins, etc.

γ-Aminobutyric acid
GABA is regarded as a non-protein amino acid found in plants and animals. GABA content is synthesized through diverse pathways in plant called ‘gaba shunt’. It was discovered in plants for half a century ago. Primarily, it is synthesized from l-glutamic acid through glutamate decarboxylase (GAD), a pyridoxal s-phosphate controlled enzyme responsible for the transformation of l-glutamic acid to GABA (Bown and Shelp, 1997). More so, GABA’s role is to act as an important depressive neuro-transmitter in the nervous system of mammals and it can also increase insulin secretion from pancreas and regulate blood pressure and heart rate to relieve pain and anxiety, respectively (Adeghate and Ponery, 2002).

Germination has proven to increase GABA content in edible seeds such as adzuki beans, kidney beans, lentil, lupin, sesame seeds, soybeans, pea, brown rice, buckwheat, waxy wheat, and oat (Gani et al., 2012; Gan et al., 2017). GABA content was significantly increased in 18 rice varieties immersed in distilled water at 30°C for 72 h, although an increase of this compound in GABA widely differed among the 18 rice varieties (Roohinejad et al., 2009, 2010). Bown and Shelp (1997) reported that GABA synthesis could be elevated by environmental stress including mechanical and environmental stimulations such as hypoxia, darkness, heat, shock, cold shock, and cystolic acidification. Chung et al. (2009) and Dewar et al. (1997) who reported that hypoxia occurs due to the limited availability of oxygen in water during seed germination result in

Figure 1. Processing of edible seeds to increase phytonutrient contents.
stress and then GABA content may increase rapidly in the seed in response to hypoxia. In addition, Lin et al. (2015) studied the effect of different germination condition on rice and reported that GABA content increases by 15-fold in a close vessel (limited oxygen) from 0.79 mg/100 g of Taiwan japonica brown rice to 12.37 mg/100 g.

Buckwheat sprouts and germinated soybean have been reported to improve GABA from 0.50 to 2.60 and from 0.60 to 37.5 (Martinez-Villaluenga et al., 2006; Lin et al., 2008). In addition, Saikusa et al. (1994) studied the effect of soaking on eight varieties of japonica rice and two hybrid varieties of indica and reported that GABA content was nearly 8 times increased.

Vitamins
Vitamins are organic compounds that are found in both plant and animal sources. They perform vital roles in human health. Conventionally, they are divided into fat-soluble and water-soluble vitamins. The water-soluble vitamins include vitamin B and C, whereas the fat-soluble vitamins include A, D, E, and K. Recent studies have proven that significant increase in the content of some vitamins can be observed as a result of germination.

Vitamin B members comprise of vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pyridoxine), vitamin B6 (folate), and vitamin B12 (cobalamin). They all have possess vital roles in human health (Pereira and Vicente, 2013). Increase in some vitamins B observed in different edible seeds has been traced to germination.

Shohag et al. (2012) reported significant increase in folate content of soybean and mung bean sprouts when compared with raw seeds by 65%–274% and 78%–326%, respectively, after germination. Also vitamins B9 and B12 increased to about 11.8 mg/100 g dry weight in buckwheat sprouts, whereas it was not detected in raw seeds (Kim et al., 2004).

Ascorbic acid (Vitamin C) is derived from fruits and vegetables. Recent studies have proven that germination can increase the content of vitamin C in edible seeds such as buckwheat, lupin, mung bean, soybean, chicken pea, and cowpea when germinated. Previous study reported by Gan et al. (2016) showed that vitamin C content of the green and black mung bean sprouts increased from 13.5 to 24.0 and 10.3 to 21.3 folds when compared with their respective raw seeds after germination for 1–5 days. Accumulation of vitamin C in germinated edible seeds can be newly formed because most seeds have low or non-availability of vitamin C before germination. Furthermore, l-galactono-gamma-lactone dehydrogenase (GLDH) is a major enzyme in ascorbic acid biosynthesis and it helps catalyse the oxidation of l-galactono-1,4-lactone to ascorbic acid. The activity of this enzymes has been observed to increase during soybean germination in parallel with the increase in ascorbic acid content (Wheeler et al., 1998; Xu et al., 2005).

Tocopherols (vitamin E) are fat-soluble vitamins which have four isomers, namely, α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol. Germination has proven to emphatically alter the content of vitamin E isomers in edible seeds. However, the mechanism is still a mystery.

β-Tocopherol, a crucial vitamin E in several edible seeds, has been reported to significantly increase in germinated soybean by 1.55%–164% according to the result of Fernandez-Orozco et al. (2008) when compared with raw seeds, but the vitamin E content in germinated lupin and mung bean reduced (Frias et al., 2005). In general, germination of edible seeds is a useful way to increase vitamins, especially vitamin C which became beneficial in human diets.

Polyphenols
Polyphenols are regarded as a group of small molecules with at least one phenol unit in their structure (Frias et al., 2005). Polyphenols exist in free or bound forms in the plant kingdom. Soluble phenolics are mostly synthesized in the intracellular endoplasmic reticulum of the plants and are stored in the vacuoles while the insoluble phenolics are found in cell wall matrix of plant cells (Agati et al., 2012). The soluble form can be extracted with water, acid, alkali, and enzymatic hydrolysis to quantify their contents in edible plant seeds. Meanwhile, germination has shown through recent studies to alter the level of total phenolics in germinated edible seeds. Gan et al. (2016) reported an increase of 5.0–5.5 folds in phenolic compound of mung bean after 5 days of germination. Increase in soluble phenolics during germination can be attributed to synthesis and conversion of different phenolic compounds (Randhir et al., 2004; Wu et al., 2012; Kim et al., 2013). Glucose is the main pre-cursor for the synthesis of phenolics compounds and several paramount molecular signalling pathways, some of which include glycolysis, propanoic pathway, hydrolysable tannin pathway, oxidative pentose phosphate pathway, and acetate/malonate pathway. They partake in the synthesis and conversion of different phenolic compounds.

Polyphenolic compounds have been less explored and reported in germinated seeds. Despite the fact that some germinated edible seeds especially cereal grains contained a lot of bound phenolics (Ti et al., 2014). In addition, some studies reported that bound phenolic first decreased and then increased after germination (Tang et al., 2014). Although in germinated brown rice, it was suggested that bound phenol content was dependent on conjugation rate and their release.

Germination at an early stage often leads to degradation of carbohydrates and protein followed by an increase in free amino acids and simple sugars and the bound phenolics conjugated with the cell wall components (Wang et al., 2005). Increase in germination time often leads to the proliferation of new plant cells as well as formation of new cell wall. Thereafter, the synthesized soluble phenolics can be secreted to the cell wall which form new bound phenolic. The known polyphenolic compounds found in germinated edible seeds are phenolic acids such as ferulic acid and coumarinic acid.

Generally, phenolic acids are found in fruits and vegetables. Many researchers reported that some germinated edible seeds contained total phenolic content ranged 30–253 mg gallic acid equivalent/100 g fresh weight, whereas common fruits contained about 11.9–386 mg gallic acid equivalent/100 g fresh weight (Lin and Tang, 2007).

Other bioactive compounds
There are other bioactive compounds reported in germinated edible seeds, apart from those listed above. Gamma oryzanol is also a bioactive compound that contains a set of 10 or more compounds with ester bonds between ferulic acid and triterpenes.

Cyloartenyl ferulate, 24- methylene cycloartenyl ferulate, and campestanyl ferulate are the major compounds of gamma oryzanol in a germinated brown rice (Jayadeep and Malleshi, 2011). Gamma oryzanol is associated with decreasing plasma and serum cholesterol (Gerhardt and Gallo, 1998). In addition, oryzanol has also been used to treat hyperlipidaemia, a disorder of menopause in women (Gani et al., 2012).

Numerous phytosterol plants have been found, among which campesterol, beta sitosterol, and stigmasterol are known to be most prevalent (Beufau et al., 2008). Germination of red rice, brown rice, glutinous brown rice, and black rice increased the stigmasterol...
levels in all the rice types tested, whereas sitosterol and campesterol showed no significant changes (Jung et al., 2013). Phytoestrogens have been used as nutritional supplements and bio-functional ingredients in foods because it can reduce the cholesterol level, prevent stroke, and display anti-atheromatosis effect (Brufau et al., 2008). Another one is melatonin (N-acetyl-5-methoxytryptamine), which is an indolamine found in plants, animals, bacteria, and fungi. It performs vital physiological functions in various organisms which includes regulation of the circadian rhythm and growth (Gamble et al., 2014). New studies have shown that germination can promote the amount of melatonin in edible seeds, e.g., germinated lentils and kidney beans.

Melatonin reached the highest content of about 2.50 ng/g dry weight and 9.50 ng/g dry weight for lentils and kidney beans, respectively, after germination for 6 days under the dark condition (Aguilera et al., 2015).

D-chiro-inositol (Dcl), a co-enzyme of glycosylphosphatidyl inositol protein, is involved in the insulin signalling pathway and glucose movement; hence, it is regarded as a pivotal insulin mediator with anti-diabetic effect (Adams et al., 2014). During germination, the content of Dcl can be promoted in germinated edible seeds. For instance, Dcl content increased gradually in mung bean by 74% (4.79 mg/g dry weight) when germinated for 80 h and decreased afterwards (Yao et al., 2011).

Conclusion

With the growing phytonutrient deficiencies in human health in many countries across the globe despite the numerous edible seeds available, it is expedient that the process of germination can be employed to improve phytonutrients in these seeds. GABA, vitamins, polyphenols, and other phytochemicals have been reported to increase during germination and has further enhanced the nutritive value of edible seeds around the globe. Thus, germination is undoubtedly an effective way to improve phytonutrients in edible seeds.

Acknowledgements

Acknowledgement goes the Higher Education Research Promotion and the Thailand’s Education Hub for Southern Region of Asian Countries Project office of the Higher Education Commission for the scholarship granted to pursue graduate studies.

Author’s Contributions

Anthony Temitope Idowu is a research assistant at Prince of Songkla University, Thailand. He conceptualized the research idea and wrote the article. Oladipupo Odunayo Olatunde, Ademola Ezekiel Adekoya, and Solomon Idowu all provided the necessary guide and correction of the article.

Conflict of Interest Statement

None declared.

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