The effect of long germination on the levels and types of non-enzymatic antioxidants in red kidney bean sprouts

Hery Winarsi[1*], Yudhanti Putri Bestari[1], Nisa Ilmi Lulu Masluki[1], Aisyah Tri Septiana[2]

[1]Department of Nutrition Science, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia
[2]Department of Food Technology, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia;

*Email: winarsi12@gmail.com

Abstract: This study aims to explore the content of several types of non-enzymatic antioxidants in red kidney beans sprout based on duration germination. The complete randomized design study was carried out by red kidney beans sprout with germination duration variables of 0, 10, 20, and 30 hours. Red kidney bean sprouts are blended with water 8 times the amount, then filtered, so that red kidney bean sprouts extract is obtained. The phenolic content determinate using the Folin-Ciocalteu method; vitamin C testing using Iodometry; and fiber content by the proximate method. Data were analyzed using ANOVA, followed by the DMRT test if there was any significance with an error rate of 5%. The selection of products with the highest antioxidant content uses the effectiveness index test. The duration of germination increased the content of phenolic antioxidant ($P < 0.05$), vitamin C ($P < 0.05$), and fiber ($P < 0.05$). Red kidney bean sprouts resulting from germination for 30 hours have the highest content antioxidants of phenolic, vitamin C, and fiber, respectively 583.85 mg GAE/L, 23.14 mg/100 mL, and 6.75%. Red kidney bean sprouts can be used as an ingredient of antioxidant-rich products that are useful for the prevention of degenerative diseases.

Keywords: antioxidant, fiber, phenolic, red kidney bean sprout, vitamin C

1. Introduction

The incidence of degenerative diseases increases over time. There are various triggering factors for the emergence of degenerative diseases, but the most important is oxidative stress [1], which is an imbalance between antioxidant status and the formation of oxidants in the body. There are 2 groups of antioxidants, namely enzymatic and non-enzymatic antioxidants [2]. Especially for non-enzymatic antioxidants are found in various foodstuffs and various forms of compounds, such as phenolic, vitamin C, fiber, vitamin E, carotenoids, lipoic acid, and others. The food rich in antioxidants can inhibit the emergence of degenerative diseases [3]. Mechanism of non-enzymatic antioxidant systems, namely by cutting the chain of oxidation reactions, so free radicals cannot react with cellular components. Nuts, besides being known as a source of vegetable protein, are also rich in antioxidants [4].

Kidney beans are said to be recommended as diets for diabetics because of their high fiber content (4%), protein (29.1%), unsaturated fatty acids, and vitamin E [5]. These beans also contain a large number of phytochemical compounds, including phenolic, flavonoids [6], as well as vitexin and isovitexin [7]. The highest antioxidant activity compared to other types of beans [8]. In general, polyphenol-rich beans are proven to protect LDL-c against oxidation, and reduce LDL absorption [9]. *Phaseolus vulgaris* L. also contains resistant starch, vitamins, and fructooligosaccharides [10] (Ambigaipalan et al. 2011) which have been proven to protect the body from oxidative stress, heart disease, diabetes, cancer, and metabolic syndrome. Wells et al. [11] reported that unsaturated fatty acids (n-3), vitamins, dietary fiber, and L-arginine play an important role in the modulation of inflammation. As a source of fiber, legumes are reported to be beneficial to human health [12]. However, red kidney beans also contain anti-nutritional compounds, such as protease inhibitors, lectins, saponins, vicine, convincing, phytate, and alkaloids, as well as indigestible oligosaccharides, such as raffinose, stachyose, verbascose, and ajugose [13].

Anti-nutritional compounds are now in great demand by researchers because they are related to their antioxidant capacity, as scavengers of free radicals and chelating metals, so that it has implications for health benefits [14][15]. The existence of anti-nutritional compounds that are similar to bioactive compounds are interlocked with other compounds in nuts, so it needs an effort to explore them.

Germination is a catabolism/breakdown process, associated with increased secretion and activates of hormones and enzymes, which hydrolyzes food reserves, transports dissolved food and hormones to the growing point, and assimilation (photosynthetic). Protease immediately converts protein molecules to amino acids; starch deposits are broken down by carbohydrases to maltose then to glucose; the fat content is broken down by lipases into fatty acids [16]. Therefore, the content of amino acids, fatty acids, and glucose of red bean sprouts is higher than red kidney beans that are not germinated, as well as the content of micronutrients and bioactive substances. However, many factors affect the success of the germination process, one of which is the duration of germination. The problem is, how long is the red kidney bean sprout to obtain the highest antioxidant content, especially antioxidants of phenolic, vitamin C, and fiber in it? This study aims to determine the effect of long duration of red kidney bean germination on the antioxidant content of phenolic, vitamin C, and fiber, and to get the right time to get the highest content of these types of antioxidants.

2. Materials and Methods

This study uses a non-factorial Complete Randomized Design with 1 type of treatment (duration of germination) and consists of 4 levels, namely 0, 10, 20, and 30 hours, with 5 repetitions, so that there were 20 units. The independent variable was long germination, while the dependent variable is the content of phenolic, vitamin C, and fiber.

2.1. Making Red Kidney Bean Sprouts[17]

Red kidney beans are sorted, washed with running water, and then soaked for 12 hours. After soaking, the red beans are drained and placed in a basket and covered with a dark cloth. Red kidney beans are incubated at room temperature with various variations of long germination, *i.e.* 0, 10, 20, and 30 hours. Each red kidney bean sprouts were weighed, then crushed using a blender with a ratio of water : red kidney bean sprouts = 8: 1. After that, filtered with a filter cloth to get the essence of red kidney bean sprouts.

2.2. Determination of phenolic content of red kidney bean sprouts

Making gallic acid calibration curves with the Folin-Ciocalteu phenol reagent [18]. First, make a gallic acid main solution utilizing 1 g of gallic acid added with 95% methanol to 100 ml to produce a concentration of 1000 ppm. From the main liquor is taken of 2.5, 3, 3.5, 4 ml, and then added to 10 ml of methanol so that the resulting concentration of 200, 250, 300, 350, and 400 mg/L gallic acid. From each of the above concentrations, 0.1 ml was added with 5 ml of distilled water and 0.2 ml of the folin-ciocalteu reagent was diluted with distilled water with a ratio of 1: 1, then shaken. Let stand for 5 minutes added 1 ml of 5%

Na_2CO_3 solution and vortex. Let stand for 1 hour in a dark condition. One of the concentrations of the standard solution is measured by its absorbance with a wavelength of 747 nm to obtain the maximum wavelength. Measure the absorbance of all standard solution concentrations with a specified maximum wavelength, then a calibration curve is made between the concentration of gallic acid (mg/ml) and the absorbance.

A sample of 0.1 ml was taken into a test tube and 5 ml of distilled water was added, and 0.2 ml of the Folin-Ciocalteu reagent was diluted with distilled water in a ratio of 1: 1, shaking. Let stand for 5 minutes added 1 ml of 5% Na_2CO_3 solution and vortex. Let stand for 1 hour in a dark condition. Measure the absorbance at the maximum wavelength that has been obtained that is 747 nm and read 2 times, do 5 repetitions [29]. Calculation of total phenolic content using the following formula as below.

$$\text{Total Phenolic Content (TPC)} = (C.V.Fd) / W$$

Note: C, phenolic concentration; V, the extract volume used (ml); Fd, dilution factor;
W, the weight of the sample used

2.3. *Determination of vitamin C content of red bean sprouts (Iodometri method*

Weighed of 20 g of slurry put into a 100 mL flask and added distilled water until the boundary mark then shaken until homogeneous. Then the solution is centrifuged and filtered) [19]. The filtrate obtained is ready to be analyzed for its vitamin C levels. A total of 10 mL of filtrate was added to the titration flask and 2 mL of 1% starch and 20 mL of distilled water were added. Then the solution is titrated with 0.01 N standard I_2 solution until a dark blue color does not disappear for 1 minute. Titration is done 3 times. The vitamin C content of red kidney bean sprouts is determined by the following formula.

$$1 \text{ mL } I_2 = 0.88 \text{ mg of Vitamin C.}$$

2.4. Determination of Fiber Content of Red Kidney Beans Sprout

First, the material is weighed 10 g of sample and then put into 600 ml Erlenmeyer. After that, 200 ml of H_2SO_4 0.3 N was put into Erlenmeyer. Then for 30 minutes simmer with low heat and sometimes the shaking Erlenmeyer so that the solution is mixed evenly Then, filter the suspension through filter paper, and the residue left in the Erlenmeyer is washed with boiling distilled water. Then, the residue in filter paper is also washed with distilled water until the washing water is no longer acidic (test with litmus paper). After the residue has been filtered, the residue is transferred quantitatively from filter paper into an Erlenmeyer with a spatula. And the rest is washed by adding as much as 200 ml of 0.3N NaOH solution that has been boiling until all the residue goes into the Erlenmeyer. Then boil again while sometimes Erlenmeyer shake it. Then filtered through a heated Gooch crucible and known for its weight by using a suction pump washed again with boiled aquades and then with ± 15 ml of 95% alcohol. After that crushes with the contents dried at 110^0C to a constant weight (for approximately 1 to 2 hours), then cooled in a desiccator and weighed, then the weight of residue which is the weight of crude fiber is obtained [20]. Fiber content is calculated using the following formula.

$$\text{Crude Fiber Content} = (\text{weight of crude fiber (g)}) / (\text{sample weight (g)}) \times 100\%$$

2.5. Data analysis

Phenolic, vitamin C, and fiber levels were analyzed using the F test at a 95% confidence level with the statistical application of SPSS 16.0. If there is a difference continue with Duncan's Multiple Range Test (DMRT) with an error of 5%. To find out the best antioxidant content based on phenolic levels, vitamin C, and fiber were tested using an effectiveness index [21] (DeGarmo et al. 1984).

3. Results and Discussions

3.1. Effects of the duration of germination on phenolic levels of red kidney bean sprout

The duration of germination had a very significant effect on the total phenolic levels of red bean sprouts (p = 1.41E-10) (Table 1). The longer the germination time, the higher phenolic content, and the highest level is found in germination with 30 hours of germination. In line with the opinion of [22] that phenolic compounds can be produced in beans which undergo elicitation during the germination process. The elicitation process is the process of the formation of phytoalexin as a defense system in plants to avoid interference with microorganisms. Synthesis of phenolic compounds as phytoalexin in the elicitation process is also driven by the presence of polysaccharides [19]. Other research also states that germination can increase the production of phenolic compounds in lupine seeds [23]. This is a natural reaction to protect plants from biotic pressures such as diseases, insects, and environmental stress.

Table 1. The content of antioxidant phenolics, vitamin C, and fiber of red kidney bean sprout based on germination time

Long germination (hour)	Phenolics (mg GAE/L)	Vitamin C (mg/100 mL)	Fiber (%)
0	240.46 ± 0.0^a	15.38 ± 1.07^a	1.83 ± 0.82^a
10	436.67 ± 6.44^b	15.56 ± 3.07^a	3.87 ± 1.52^b
20	513.08 ± 5.47^c	16.4 ± 3.18^a	5.99 ± 3.75^b
30	583.85 ± 10.49^d	23.14 ± 3.83^b	6.75 ± 1.6^c

Notes: N=5; Numbers with similar letters on column show a non-significant difference (p>0.05).

The researchers stated that germination can increase the total levels of phenolic coma beans because during the germination process there is the biosynthesis of plant hormones to produce phenolic compounds. Xu et al. [24] reported that free phenolics and avenanthramides are the main phenolic components of wheat, increasing during germination. Phenolic levels were also reported to increase significantly during germination in the legume [25]. Increased levels of phenolics in pulses (beans, white beans, lentils, *Vicia sativa* L) during germination, are associated with increasingly active endogenous enzymes, such as hydrolase and polyphenol oxidase [26].

Biosynthesis of phenolic compounds is on the same path as the biosynthesis of growth hormones, such as auxin, gibberellins, and cytokines. Before being germinated with red beans, it must be soaked for 12 hours. When immersion occurs the imbibition process is the process of absorption of water. Water absorption causes kidney beans to contain enough water to activate the growth hormone. This results in phenolic biosynthesis in red kidney beans that have not germinated (0 hours) used for sprout growth, so that phenolic levels are still low. Furthermore, in the germination period of 10 hours to 20 hours, the total phenol content increased, even reaching its peak at 30 hours. Chances are, growth hormone biosynthesis is used for the production of phenolic compounds.

The results of this study are by the findings of [27], which stated that there was a 10% increase in flavonol and flavonon levels in red beans after the germination process compared with red bean seeds without germination process. Likewise, Zhang et al. [28] added that the longer the germination process affects the phenolic increase in Buckwheat after going through the germination process for 36, 48, and 72 hours.

Increased levels of phenolic can also occur due to the stimulation of the biosynthesis of phenolic compounds through the shikimate acid pathway. Phenolic acid, flavonoids, and tannins are secondary metabolites that are very important during plant growth. These compounds are synthesized through the shikimate pathway and the phenylpropanoid biosynthetic pathway. In the process of growth, L-phenylalanine is converted to cinnamic acid which is catalyzed by phenylalanine ammonia-lyase (PAL) [28]. In this metabolic pathway, PAL is an important enzyme for the biosynthesis of flavonoids and phenolic acids. Tang and Zhao [30] and Kong et al. [31] stated that PAL activity increases during germination. Thus it can be assumed that the increase in phenolic levels of red bean sprouts is due to increased PAL activity during the germination process.

3.2. Effects of the duration of germination on vitamin C levels of red kidney bean sprouts

Germination increases vitamin C levels, but a significant increase occurs at 30 hours of germination ($P = 0.005$) (Table 1). The biosynthetic pathway of vitamin C in plants has been reviewed by several researchers. Vitamin C is a compound that is generally needed in most living organisms because it plays an important role in scavenging free radicals in living cells. The L-galactose pathway is the main pathway for the biosynthesis of vitamin C (L-ascorbic acid) in dicotyledonous plants [32]. This process begins with D-glucose phosphorylation by hexokinase and ends with epimerization of L-Galactono-1,4-lactone to L-ascorbic acid by L-galactono-1,4-lactone dehydrogenase. Lay and Fields [33] add that germinated seeds have a much higher ascorbic acid content, compared to seeds that are not germinated. Likewise, the opinion of Huang et al. [34], that no vitamin C was found in soybeans and green beans that did not germinate, but their levels increased during germination. Similarly, the findings of [35] that vitamin C is not found in bean seeds, but the vitamin began to be found on the first day of germination to reach 30 mg per 100 grams after 48 hours.

Germination can also significantly increase vitamin C content in a variety of seeds, such as wheat, beans, cowpea, lupine, green beans, and soybeans [14]. It was also mentioned that green beans and black beans, their vitamin C levels increased from 13.5 to 24.0 and 10.3 to 21.3 times compared with the original seeds, after experiencing germination for 1 - 5 days. The accumulation of vitamin C in germinated seeds is caused by de novo synthesis, because most seeds have very low vitamin C content, before germination. This is because the activity of the main enzyme in the biosynthesis of ascorbic acid, L-galactono-lactone dehydrogenase, increases markedly during germination [24]. The enzyme L-galactono-act-lactone dehydrogenase catalyzes the oxidation of L-galactono-1,4-lactone to ascorbic acid, so the longer the germination time can increase the levels of ascorbic acid in red bean sprouts.

Gorelova et al.[36] states that folate metabolism and total NADPH synthesis that occurs in animals, may also occur in plants. NADPH is involved in the ascorbate-glutathione cycle, which plays an important role in removing ROS by reducing monodehydroascorbate and subsequently becoming ascorbic acid. Therefore, the accumulation of folate in sprouts can lead to accumulation of NADPH, and subsequently contribute to the increased content of ascorbic acid or vitamin C.

3.3. Effects of the duration of germination on the fiber content of red kidney bean sprouts

As the length of germination increases the higher the fiber content in red kidney bean sprouts ($P<0.05$) (Table 1). Crude fiber consists of cellulose, hemicellulose, and lignin. Nearly 50% of carbohydrates derived from plants are cellulose, which is the most important part of plant cell walls. According to [37], an increase in the amount of cellulose in metabolic reactions as well as germination. Nkhata et al. [38] stated that hydrolysis by microbes from cellular materials such as proteins, fats, and carbohydrates can increase fiber, therefore increasing the cellular structure of plants during germination also allows increased fiber content.

The highest fiber content was found in red bean sprouts with 30 hours germination time which was 6.75%, while the lowest was in 10 hours germination which was 3.87%. The results of this study are in line with the opinion of the following researchers that germination can increase the levels of crude fiber, so that food fiber also increases. Uppal and Bains [39], showed that fiber levels in beans, mung beans, and cowpea experienced a significant increase during the germination process. Megat et al. [40] also proved that red beans, green beans, soybeans and cooking beans increased their fiber content significantly during germination, so that the bean sprouts can be used as functional ingredients in milk products.

High levels of fiber in red kidney bean sprouts are very good for health. Li and Komarek [41] confirm that high fiber consumption is a safe and practical effort to reduce blood cholesterol levels. Dietary fiber cannot be absorbed and digested by enzymes in the human intestine, but it can affect the absorption of water in the digestive system, thereby increasing the volume of food in it, and causing a feeling of fullness longer. Recent studies have shown that fiber can help reduce blood glucose and triglyceride levels, as well as blood cholesterol [42]. Fiber can be fermented in the intestine to produce short-chain fatty acids (SCFA), including propionate which can prevent an increase in cholesterol, and butyrate which can prevent colon cancer. Fiber can bind to organic molecules that cause the binding of bile so that cholesterol decreases.

3.4. The red kidney bean sprouts rich in antioxidant phenolics, vitamin C, and fiber

Henceforth, to find out how long the germination produces red kidney bean sprouts with the highest antioxidant content, an effectiveness index test according to [21]. Based on the effectiveness index test at Table 2 with the weighting parameters of phenolic antioxidants was 1, vitamin C was 0.9, and fiber was 0.8, obtained red kidney bean sprouts with the highest antioxidant content of phenolic, vitamin C, and fiber was 30 hours germination (Table 2), *i.e.* red kidney bean sprouts with 583.85 mg GAE/L phenolic, 23.14 mg/100 mL vitamin C, and 6.75% fiber. Some researchers report that antioxidant-rich products are beneficial for health.

Phenolic is an antioxidant that has the potential to protect LDL-c against oxidation (LDL-OX) and reduce LDL absorption [9]. LDL-ox which accumulates, forms plaque, clogging arteries, causing atherosclerosis. Therefore phenolic antioxidants can prevent atherosclerosis, which starts coronary heart disease.

Phenolic, one of the phytochemicals is antioxidant and has the potential to be anti-inflammatory. Phenolic in red bean sprouts has been shown to suppress inflammation by $23.02 \pm 2.3\%$ in carrageenan-induced white rats [43], as stated by [44] that phenolics are also proven as anti-inflammatory. As a potential antioxidant, phenolic can improve the antioxidant and immune status of women with oxidative stress. Thus phenolic rich red bean sprouts are beneficial for sufferers of degenerative diseases due to oxidative stress.

Vitamin C antioxidants have been shown to protect the body from oxidative stress, heart disease, diabetes, cancer, and metabolic syndrome [45]. The Mayo Clinic attempts to repeat the results of Pauling and Cameron in a randomized placebo-controlled study of patients with advanced cancer, with oral administration of 10 g of vitamin C per day to exclude clinical symptoms. Vitamin C can provide clinical benefits with high intravenous doses, and is given as a program for months. The phase I trial showed that high doses of vitamin C were a useful addition to chemotherapy [46].

Vitamin C is a free radical scavenger that dissolves in water. Besides, the vitamin regenerates vitamin E in cell membranes by combining with GSH or compounds that can contribute to reducing equivalents. Changes in vitamin C, become ascorbate radicals after donating electrons to lipid radicals to end the lipid peroxidation chain reaction. The radical pair of ascorbate reacts quickly to produce one molecule of ascorbate again and one molecule of dehydroascorbate. Dehydroascorbate does not have antioxidant capacity. Therefore, dehydroascorbate is converted back to ascorbic acid by the addition of two electrons. The last step (the addition of two electrons to dehydroascorbate) is carried out by oxidoreductase [47]. Vitamin C reported by [48] also showed its potential in reducing inflammation by reducing levels of hs-CRP, IL-6, and fasting sugar levels. Similarly, antioxidant-rich products of vitamin C are beneficial for people with cancer, diabetes, and others.

Table 2. The effectiveness of red kidney bean sprouts

Parameters	VW	NW	The duration of germination (hour)							
			0		10		20		30	
			VE	VP	VE	VP	VE	VP	VE	VP
Phenolics	1	0.37	0	0	0.21	0.08	0.29	0.11	0.37	0.14
Vitamin C	0.9	0.33	0	0	0.01	0.00	0.04	0.04	0.33	0.11
Fiber	0.8	0.30	0	0	0.12	0.04	0.25	0.07	0.30	0.09
VW	2.7									
Total VP				0		0.12		0.22		0.34

Notes: VW, variable weights, NW, Normal Weight, VE, Value of Effectiveness, VP, Value of Product.
Calculation:

1. Add up all the values of the variable weights (VW): (1+0.9+0.8)= 2.7
2. NW = VW/ Total amount of VW
3. Value of Effectiveness (VE) = (Average treatment value) / (Lowest average value)
4. VP = VE x NW
5. Add all of NPs where the biggest yield is the best treatment

Associated with fiber antioxidants, Singh et al. [49] confirms that fiber is a non-nutritional compound that can fight cancer and keep cholesterol and blood sugar normal. The effect of fiber in controlling blood sugar levels, possibly by reducing energy intake and the glycemic index is expected to be achieved through food expansion. Energy reduction can reduce energy density from food intake, while food expansion prevents further food intake. Qi et al. [50] discovered the hypoglycemic effect of fiber through other mechanisms. It has been observed that rat feed intake did not differ between the fiber group and the animal feed control group with the same energy density. Thus, the confounding factors of energy reduction and food expansion can be eliminated. Fiber significantly reduced blood glucose levels and visceral fat levels in rats, compared to rats in the control group [51].

Fiber has a variety of mechanisms of action, depending on the source and the amount of fiber consumed. Fiorucci and Distrutti [52] argue that fiber provides physiological effects in humans through several mechanisms such as increasing the excretion of bile acids and neutral steroids through feces. Fiber is also able to change the ratio of primary and secondary bile acids, and increase the excretion of cholesterol and fatty acids through feces. Indirectly, by consuming foods high in fiber, as well as removing body fat and cholesterol, so cholesterol levels decline, and improve lipid profile. Kidney fiber sprouts are beneficial for the health of people with dyslipidemia. Thus the sprouts are rich in phenolic antioxidants, vitamin C, and fiber are very useful for the prevention of degenerative diseases.

4. Conclusion

The conclusion, that long germination increases the content of various antioxidants, such as phenolic, vitamin C, and fiber in red bean sprouts. The highest levels of antioxidants are found in red beans that are germinated for 30 hours. The high antioxidant in red bean sprouts makes it possible as a component of an antioxidant-rich product, which is very beneficial for sufferers of degenerative diseases associated with oxidative stress.

Acknowledgement

The authors would like to express their gratitude to the Directorate General of Development and Research Enhancement of the Ministry of Research, Technology, and Higher Education of Indonesia, who has funded this study through the Competency-Based Research scheme with contract number 632/UN23.18/PN.01.05/2020. 10 Maret 2020.

References

[1] Bhat AH, Dar KB, Anees S, Zargar MA, Masood A, Sofi MA, Ganie SA. 2015. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. Biomed Pharmacother 74: 101-110. doi:10.1016/j.biopha.2015.07.025

[2] Winarsi H. 2007. Antioksidan Alami dan Radikal Bebas. Kanisius, Yogyakarta

[3] Tan BL, Norhaizan ME, Liew WPP, Sulaiman, Rahman H. 2018. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front. Pharmacol. 9. doi:10.3389/fphar.2018.01162

[4] Oboh G. 2006. Antioxidant properties of some commonly consumed and under utilized tropical legumes. Eur Food Res Technol 224(1): 61-65.

[5] Hayat I, Ahmad A, Masud T, Bashir S. 2014. Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): An overview. Crit Rev Food Sci Nutr 54: 580-592.

[6] Harada G, Kondo N. 2009. Adzuki bean leaf infection by *Phytophthora vignae* f. sp. adzukicola and resistance evaluation using detached leaves inoculated with zoospores. J Gen Plant Pathol 75(1):52-55.

[7] Luo J, Cai W, Wu T, Xu B. 2016. Phytochemical distribution in hull and cotyledon of adzuki bean (*Vigna angularis* L.) and mung bean (*Vigna radiate* L.), and their contribution to antioxidant, anti-inflammatory and anti-diabetic activities. Food Chem 201: 350-360.

[8] Kiyani-Sam M, Amjad L, Ranjbar M. 2015. Comparative study of antioxidant activity, phenol and flavonoid content in *Phaseolus vulgaris* and *Vigna sinensis* in different stages of seed germination. Int. J. Biosci. 6(5): 292-297.

[9] Winarsi H, Wijayanti SPM, Purwanto A. 2012. Soy germ protein with or without-Zn improve plasma lipid profile in metabolic syndrome women. Hayati J Biosci 19(1): 25-30.

[10] Ambigaipalan P, Hoover R, Donner E, Liu Q, Jaiswal S, Chibbar R, Nantanga KKM, Seetharaman K. 2011. Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. Food Res. Int. 44: 2962-2974.

[11] Wells B, Mainous A, Everett C. 2005. Association between dietary arginine and C-reactive protein. Nutrition 21: 125-130.

[12] Tosh SM, Yada S. 2010. Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. Food Res Int 43(2):450-460. doi:10.1016/j.foodres.2009.09.005

[13] Haileslassie HA, Henry CJ, Tyler RT. 2016. Impact of household food processing strategies on antinutrient (phytate, tannin, and polyphenol) contents of chickpeas (Cicer arietinum L.) and beans (Phaseolus vulgaris L.): a review. Int J Food Sci Tech. 51(9):1947-1957. Doi:10.1111/ijfs.13166

[14] Gan R-Y, Lui W-Y, Wu K, Chan Ch-L, Dai S-H, Sui Z-Q, Corke H. 2016. Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. Trends Food Sci Tech. DOI: 10.1016/j.tifs.2016.11.010

[15] Gulewicz P, Martinez-Villaluenga C, Kasprowicz-Potocka M, Frias J. 2014. Non-nutritive compounds in Fabaceae family seeds and the improvement of their nutritional quality by traditional processing–a review. Pol J Food Nutr Sci 64(2): 75-89

[16] Chaudhary N, Vyas S, Joshi I. 2013. Biochemical and enzymatic changes associated with duration of germination of wheat moth based food mixes. Int. J. Sci. Res 1: 2319-7064

[17] Nurjanati M, Winarsi H, Dwiyanti H. 2018. The effect of germination time on sensory properties d soluble protein content in red beans sprouts milk for obese adolescents. J. Gipas, 2(2): 27-42.
[18] Waterhouse A. 1999. Folin - Ciocalteau Micro Method For Total Phenol In Wine, Department Of Viticulture & Enology University Of California, Davis, 152-178.
[19] Baenas N, García-Viguera C, Moreno D. 2014. Elicitation: A tool for enriching the bioactive composition of foods. Molecules 19(9): 13541-13563. doi:10.3390/molecules190913541
[20] Sudarmadji S, Suhardi, Haryono B. 2010. Analisa Bahan Makanan dan Pertanian. Liberty Yogyakarta.
[21] DeGarmo EP, Sullivan WG, Canada JR. 1984. Engineering Economy. 7th Edition, Macmillan Pub. Co, New York. 79-96.
[22] Liu H, Kang Y, Zhao X, Liu Y, Zhang X, Zhang S. 2019. Effects of elicitation on bioactive compounds and biological activities of sprouts. J. Funct. Foods 53: 136-145. doi:10.1016/j.jff.2018.12.019
[23] Dueñas M, Hernández T, Estrella I, Fernández D. 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chem 117: 599-607.
[24] Xu JG, Tian CR, Hu QP, Luo JY, Wang XD, Tian XD. 2009. Dynamic changes in phenolic compounds and antioxidant activity in oats (Avena nuda L.) during steeping and germination. J. Agric. Food Chem. 57: 10392-10398.
[25] Khang TD, Dung TN, Elzaawely AA, Xuan TD. 2016. Phenolic profiles and antioxidant activity of germinated legumes. Foods 27 (5):2-10
[26] Gharachorloo M, Tarzi BG, Baharinia M, Hemaci AH. 2012. Antioxidant activity and phenolic content of germinated lentil (Lens culinaris). J. Med. Plants Res. 6: 4562-4566.
[27] Dueñas M, Cristina MF, Rocio IL, Elena P, Juana F. 2015. Effect of germination and elicitation on phenolic composition and bioactivity of kidney beans. Food Res Int 70(1): 55-63.
[28] Zhang G, Xu Z, Gao Y, Huang X, Zou Y, Yang T. 2015. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. J. Food Sci 80(5): H1111-H1119. doi:10.1111/1750-3841.12830
[29] Orak HH. 2006, Total antioxidant activities, phenolics, anthocyanins, polyphenoloxides activities in red grape varieties. Electron. J. Pol. Agric. Univ 111(3): 235-241
[30] Tang Y, Zhao G. 1998. The relationship between phenylalanin deaminase activities and xanthoketone contens in buckwhead. J Mianyang Coll Econ Technol 15(1): 9-12.
[31] Kong WB, Fan W, Lu J, Zhao HF, Dong JJ, Shan LJ, Lin Y. 2007. Changes of phenolic compounds and related enzymes during barley malting, J Food Sci Biotechnol, 26(5): 41-50.
[32] Hanson AD, Gregory JF. 2011. Folate biosynthesis, turnover, and transport in plants. Annu. Rev. Plant Biol 62: 105.
[33] Lay MG, Fields ML. 2010. Nutritive value of germinated corn and corn fermented after germination. J. Food Sci. 46: 1069-1073.
[34] Huang X, Cai W, Xu B. 2014. Kinetic changes of nutrients and antioxidant capacities of germinated soybean (*Glycine max* L.) and mung bean (*Vigna radiata* L.) with germination time. Food Chem 143: 268-276.
[35] Wea ASY, Widodo R, Pratomo YA. 2014. Evaluation of quality of mung bean sprout product, sprout age and Na-CMC concentration study. Jurnal Teknik Industri Heuristic 11(1): 61-79.
[36] Gorelova V, Ambach L, Rebeille F, Stove C, Van der Straeten D. 2017. Folates in plants: research advances and progress in crop biofortification. Front. Chem 5: 1-20.

[37] Benítez V, Cantera S, Aguilera Y, Mollá E, Esteban RM, Díaz MF, Martín-Cabrejas MA. 2013. Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. Food Res. Int. 50: 64-69.
[38] Nkhata SG, Ayua E, Kamau EH, Shingiro J-B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr. 6: 2446-2458. doi:10.1002/fsn3.846
[39] Uppal V, Bains K. 2012. Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes. J Food Sci Technol 49(2): 184-191.
[40] Megat RMR, Azrina A, Norhaizan ME. 2016. Effect of germination on total dietary fibre and total sugar in selected legumes. Int Food Res J, 23(1): 257–261.
[41] Li YO, Komarek AR. 2017. Dietary fibre basics: Health, nutrition, analysis, and applications. Food Qual Saf, 1(1): 47-59. doi:10.1093/fqsafe/fyx007
[42] Yang Y, Lu X, Liu Q, Dai Y, Zhu X, Wen Y. 2017. Palmitoyl ascorbate and doxorubicin co-encapsulated liposome for synergistic anticancer therapy. Eur. J. Pharm. Sci. 105: 219-229. doi: 10.1016/j.ejps.2017.05.038
[43] Winarsi H, Wulandari SP, Susilowati SS, Amurwanto A. 2018. Red kidney bean sprouts milk rich in phenolic antioxidants and vitamin C has the potential as an anti-inflammatory in carrageenan-induced mice. Seminar Nasional Pengembangan Sumber daya Perdesaan dan kearifan local berkelanjutan VIII. Purwokerto. November 2018.
[44] Winarsi H, Sasongko ND, Purwanto A. 2016. Germinated-soy milk in supressing inflammation and oxidative stress in blood plasma and breast milk of lactating mothers. Int Food Res J 23(2): 646-652.
[45] Oomah BD, Corbé A, Balasubramanian P. 2010. Antioxidant and anti-inflammatory activities of bean (*Phaseolus vulgaris* L.) hulls. J. Agric. Food Chem. 58: 8225-8230.
[46] Mikirova N, Hunnunghake R, Scimeca RC, Chinshaw C, Ali F, Brannon C. 2016. High-dose intravenous vitamin C treatment of a child with neurofibromatosis type 1 and optic pathway glioma: a case report. Am. J. Case Rep. 17: 774-781. doi: 10.12659/AJCR.899754
[47] Oh Ch, Li M, Kim E-H, Park JS, Lee J-Ch, Ham SW. 2010. Antioxidant and radical scavenging activities of ascorbic acid derivatives conjugated with organogermanium. Bull. Korean Chem. Soc. 31(12): 3513 DOI 10.5012/bkcs.2010.31.12.3513
[48] Ellulu MS, Rahmat A, Ismail P, Khaza'ai H, Abed Y. 2015. Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. Drug Des Devel Ther 9: 3405-3412. https://doi.org/10.2147/DDDT.S83144
[49] Singh B, Singh JP, Shevkani K, Singh N, Kaur A. 2017. Bioactive constituents in pulses and their health benefits. Int J Food Sci Tech 54: 858-870.
[50] Qi J, Li Y, Masamba KG, Shoemaker CF, Zhong F, Majeed H, Ma J. 2016. The effect of chemical treatment on the In vitro hypoglycemic properties of rice bran insoluble dietary fiber. Food Hydrocoll, 52: 699-706.
[51] Shen L, Keenan MJ, Raggio A, Williams C, Martin RJ. 2011. Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. Mol Nutr Food Res, 55: 1499-508.
[52] Fiorucci S, Distrutti E. 2015. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. Trends. Mol. Med. 21(11): 702-714. doi:10.1016/j.molmed.2015.09.001
[53] Bekele DA, Geleta GS. 2015. Iodometric determination of the ascorbic acid (Vitamin C) content of some fruits consumed in Jimma Town community in Ethiopia. Res J Chem Sci 5(1): 60-63