International Journal of Engineering & Technology, 8 (1.4) (2019) 266-274

International Journal of Engineering & Technology

Website: www.sciencepubco.com/index.php/IJET

Research paper

Impact of Germination period on Bioactive compounds and Vitamin contents in Quinoa, Amaranth and Brown Rice

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Abstract

The present study aimed to determine changes in the activities of digestive enzymes and contents of bioactive substances and antioxidant activities in quinoa, amaranth, and brown rice according to the germination period. Grains were germinated in water for 6 h followed by sprouting in air for 6, 12, 24, and 48 h. The enzyme activities including α-amylase and protease significantly increased in proportion to the germination period, and that of germinated quinoa(GQ) was the highest in the 24 h-germinated group and that of germinated amaranth(GA), in the 48 h-germination group. The α-amylase activity of GQ and GA was significantly higher than that of the control barley malt (M) and that of the germinated brown rice(GB) was also the highest in the 48 h-germination group but the lowest among all test groups. The protease activity also increased in proportion to the germination period, and that of both GQ and GA was significantly high in the 48 h-germination group. Similarly, γ-aminobutyric acid (GABA), niacin, ascorbic acid, total poly phenol contents, and flavonoids significantly increased with respect to the germination time in Andean seeds with increase of antioxidant activity. In conclusion, optimal germination can stimulate bioactive changes in quinoa, amaranth and brown rice.

Keywords: *germination, quinoa, amaranth, brown rice*

1. Introduction

Quinoa (*Chenopodium quinoa*) and amaranth (*Amaranthus cruentus*) originated in ancient Central and South America were staple foods during the Peruvian Inca and Mexican Aztec eras [1]. These pseudocereals has attracted increasing attention by the European health food industry for health-beneficial properties [2]. Recently, quinoa and amaranth has been cultivated not only in their native areas but in other areas such as Mexico, Africa, India, part of China [1] and Korea. Since quinoa and amaranth contain macronutrients including carbohydrates, essential amino acids and unsaturated fatty acids, and phospholipids, they can be good resources of healthy calories. Furthermore, they contain adequate amounts of vitamins, minerals and cellulose for human nutrition, and the functional components, such as saponin, phytosterol, squalene, and polyphenol [3,4]. They are now used not only as ingredients of cereals and confectionery baking but also as substitute food for gluten-free diet [5].

Germination refers to the emergence of the plumule and radicle in a plant seed. In order for plant seeds to germinate, appropriate conditions of oxygen, moisture, temperatures and light must be met, and germination requires a certain amount of time [6,7]. Once plant seeds start to germinate, various enzymes contained in them begin to be activated, respiration becomes active, and metabolic responses which convert the stored nutrients of the gemmule into easy-to-use forms occur [6-8]. In the grain or seed, germination has important effects on the chemical composition, nutritive value and sensory acceptability characteristics of products for human consumption [9]. After germination, there is a decrease in the caloric content of the grains. The nutrient-energy ratio of some vitamins is higher than in the original seed [9]. It is known that when brown rice germinates, various enzymatic activities are increased, its functional ingredients and nutrients such as γ-aminobutyric acid (GABA), ferulic acid and inositol are increased, its taste is improved, its texture becomes soft, and it is made easier to digest and absorb. Studies of various physiological activities such as prevention of hypertension, anti-obesity effects, and sedative effects based on these results were reported [7,10-12]. Since α-Amylase and protease activities of germinated-barley malt are also remarkably increased during germination, malt is used in making various food products such as beer, whiskey, and starch syrup as well as Korean traditional foods including sikhye (a Korean traditional rice beverage), yeot (taffy), and Korean fermented soybean foods. In addition, it has been reported that germination of quinoa and amaranth significantly increases the polyphenol content such as total phenolic and anthocyanin contents and improves antioxidant activity [3,4,7]. Germinated rye contained substantial amounts of total phenolics having significantly higher content compared with the non-germinated rye. The potential of germinated barley, sorghum, and rye for the development of effective physiologically bioactive compounds for the reduction of the risk of diabetic agents and colon cancer based on the α-glucosidase and α-amylase activities [13]. Pitzschke *et al*. [2] recently reported that germination of quinoa in vitamin rich medium can improve the nutritional value as well as indirect benefits on the elevation of proline content and a higher antioxidant capacity. Hence, this study aimed to investigate germination-related changes of Andean seeds in the activities of starch degrading enzyme, proteolytic enzyme and lipid enzyme, and to understand the bioactive components and antioxidant activities of germinated grains through a comparison with changes in those of brown rice and barley malt, and to provide basic data for future studies of food processing and bio-industries.

2. Materials and Methods

2.1. Materials

Quinoa and amaranth were cultivated and harvested by farmhouses in Pyeongchang and Hongcheon, Gangwon-do in 2016 were purchased, and brown rice and barley malt were purchased at Nonghyup Hanaro Mart in Okcheon-dong, Gangneung-si, Gangwon-do. All other standards and reagents used for analysis were purchased from Sigma-Aldrich (USA).

2.2. Methods

2.2.1. Germinated sample preparation

Sample preparation was described in our previous study [7]. Briefly, experimental equipment was sterilized with 90% alcohol and by UV irradiation. Each grain sample, quinoa, amaranth and brown rice was rinsed 3 times with distilled water, and the water was removed. 100 g of each sample were placed in a CucKoo germinator (Cuckoo Electronics, CW-5511) and sufficiently immersed in 2.5 L of water. Each grain sample was subjected to dry (in the air) germination after wet (soaking) germination for 6 days. The germination temperature was adjusted to the chungkukjang production mode (about 38 ~ 40 ℃) of the germinator used. For the germination period of dry (space) germination, germination was proceeded with for 6, 12, 24, and 48 h, respectively. Germination states were confirmed by the stereoscopic microscope (Nikon, SMZ800), and germinated samples were frozen in a cryogenic freezer, and then dried in a freeze dryer (OPERON, FDS-12012) for 72 h. For the enzyme activity experiment, the samples in experimental groups (quinoa, amaranth, brown rice) and control group (malt) were each pulverized, 1g of each was dissolved in 10 mL of 0.5% NaCl, and extraction was performed at 20℃ for 2 h. The extract was centrifuged and the supernatant was stored at -70 ℃ in a cryogenic freezer and used as an enzyme solution.

2.2.2. α-Amylase Activity

α-Amylase activity was measured by the method of Yamamoto *et al.* [14]. 2 mL of the substrate solution (1% starch solution, pH 7.4) was preheated at 40 °C for 5 minutes, and 200 µL of the enzyme solution of each sample was added to induce enzyme reaction for 20 minutes. To terminate the reaction, 3 mL of 1 M TCA was used, and 1 mL of 0.01 N iodine solution was used as the coupler. Absorbance was measured at 660 nm. At this time, changes in absorbance were measured at constant time intervals for 30 minutes after the initiation of the iodine-starch reaction in the mixture of the starch solution and enzyme solution. When the iodine-starch reaction absorbance was decreased by 10% for 10 minutes, it was defined as 1 unit of α-Amylase activity.

2.2.3. Protease Activity

Protease activity was measured in accordance with the method of Seok et al. [15]. Using 1% casein as a substrate, 1 mL of the enzyme solution of each sample was added and reaction was proceeded at 40 °C for 10 minutes. The reaction was terminated using 3 mL of 0.4 M TCA. After incubation at room temperature for 30 minutes, 5 mL of 0.4 M Na_2CO_3 and 1 mL of 1N Folin reagent were added to 2 mL of the supernatant after removing precipitates from the supernatant. Then, after 30 minutes of color development, absorbance was measured at 660 nm. Protease activity was determined by the tyrosine content of each sample reaction solution using the calibration curve using tyrosine as the standard, and when 1 µg of tyrosine was produced for 1 minute, it was defined as 1 unit.

2.2.4. Lipase activity

Lipase activity was determined by the method of Kwon & Rhee [7, 17]. Using olive oil emulsion as a substrate, which contain olive oil 5% (v/v) and gum arabic 2% (w/v) in 10 mM potassium phosphate buffer (pH 7.0), 0.3 mL substrate was mixed into 0.2 mL enzyme solution of each sample and then reaction was proceeded with stirring at 37 ℃ for 30 minutes. The reaction was ended by the addition of 0.2 mL ethanol: acetone mixture (1:1) and 1 mL iso-octan followed by the addition of 0.2 mL cupper reagent (pH 6.1). These mixture was then homogenized (Ultra-Turrax T50 homogenizer, USA) at 12,000 rpm for 3 min. The absorbance of the supernatant was measured at 715 nm. Lipase activity was defined as 1 unit when the absorbance of the enzyme solution was increased 0.01 per 10 min.

2.2.5. Determination of γ-amino butyric acid (GABA)

The sample extraction procedure was carried out with slight modification of the reported method of Komatsuzaki *et al* [17] and Hayat *et al* [18]. The powdered sample (1 g) was placed in a falcon tube with 5 mL of 80% *(v/v)* ethanol and shaken on a vortex mixer for 5 min. The sample was centrifuged (5,000 rpm) for 10 min at 4 °C and the supernatant was filtered with Millipore filter paper (pore size 0.45 µm, diam. 47 mm). The extraction step was repeated twice. The resultant extracts were dried on a rotary evaporator till the complete evaporation of ethanol from extract and the dried residue was dissolved in 1 mL water for derivatization followed by HPLC analysis. All samples were subjected to the extraction processes and were analyzed in triplicate. 1 mL aliquots of sample extract were treated with the derivatization reagents. The standards and samples were subjected to HPLC analyses (Agilent 1200 series) with a linear gradient system of solvent A (water) and solvent B (methanol). The gradient elution procedure was 60% A + 40% B at 0 min, 50% A + 50% B at 2 min, 40%A + 60% B at 5 min 30% A + 70% B at 10 min, 25% A + 75% B at 12 min and 20% A + 80% B at 15 min. This system allowed the separation in 15 min using a C-8 reversed phase column and peak detection at 230 nm. The HPLC method was validated with respect to linearity, accuracy, precision, and selectivity. Linearity of the method was examined for GABA and BAs standards (with five replicates) of concentration ranging 0.5 µg/mL–8.0 µg/mL. Method precision was examined by the analysis of samples in triplicate and expressed as RSD%. Recovery analysis was performed by spiking a known amount of standard to the rice samples and then the recoveries of GABA and BAs were determined on HPLC with triplicates and the mean was calculated on Microsoft Excel. Selectivity of the method was examined by the calculation of resolution between two peaks of the chromatogram. LOD and LOQ for this method were determined by setting signal to noise ratio as 3:1 and 10:1, respectively.

2.2.6. Determination of thiamine and niacin

The thiamine content of samples was determined using the standard method with minor modifications. The 6 g of ground samples were mixed with 25 ml of 10% trichloroacetic acid and incubated in water bath for 60 min. The pH of the mixture was adjusted at 4.5 with 2 N Sodium acetate buffer, followed by the incubation with 300 mg of Takadiastase at 37 ℃ for 18 h. The mixture was centrifuged at 3,000 rpm for 5 min and the aqueous phase was filtered through a 0.2 ㎛ syringe filter. Then, 5 ㎕ of supernatant were injected onto LC-MS/MS (Shiseido Nanospace SI2, API 3200, AB Sciex USA). An analytical column (Unison UK-C_{18} 2 x 100 mm, 3 ㎛) was used with an isocratic solvent system consisting 0.1% formic acid in DW and 0.1% formic acid in methanol. The MS conditions were as follows: ESI positive mode, ion-spray voltage of 5.5 kV; vaporizer temperature, 500 ℃. To prepare the niacin measurement, six gram of ground samples were mixed with 25 ml of 0.1 N-HCl and incubated in water bath for 60 min followed by mixing for 5 min in shaking system. The mixture was then centrifuged at 3,000 rpm for 5 min and the aqueous phase was filtered through a 0.2 ㎛ syringe filter and then was analyzed by the same system as described above. The vitamin standards were prepared in a mobile phase. Peaks were verified by adding the standard vitamins to samples and each peak area was calculated, in relation to a standard peak area. The results were calculated as follows:

Vitamin B (mg/100g) = S x (a x b)/ sample (g) x 100/1000

S: Vitamin B contents in a sample (mg/mL)

a: total volume of test sample (mL)

b: dilution rate

2.2.7. Determination of ascorbic acid

The ascorbic acid content of samples was determined using the standard method of Korean Food Standards Codex. The 10 g of ground samples were mixed with 20 ml of 5% meta-phosphoric acid and homogenized (Ultra-Turrax T50 homogenizer, USA) at 12,000 rpm for 1 min. The aqueous phase was filtered through a Whatman No. 42 filter paper and a 0.45 µm membrane filter, sequentially. Then 10 µl of supernatant were injected into HPLC system (Spectraphysiscs, 8000, USA), in which the wavelength was set to 254 nm, in absorbance mode. An analytic column (Inertsil Diol, 4.6 x 250 mm, 5 µm) was used, with isocratic solvent system consisting of 90% acetonitril and 9.9% 10 mM sodium acetate and 0.1% trifluoroacetic acid. The flow rate was 1 ml/min. The vitamin standards were prepared in a mobile phase. Peaks were verified by adding the standard vitamin to samples and each peak area was calculated, in relation to a standard peak area.

Vitamin C (mg/100g) = (c x d)/ sample (g) x 100/1000

c: Vitamin C content in a sample (mg/kg)

d: dilution rate

2.2.8. Determination of bioactive compounds

The extraction of antioxidant compounds needed for the analysis of free radical scavenging ability on total phenolic compounds content and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was carried out with 10 g of each ground grain, 100 mL of methanol (5:5; w/v) and stirring for 6 h with a magnetic bar. The extracted samples were then filtered through Whatman No. 1 filter paper, the residue was washed with 100 mL methanol and the extracts were pooled. The extracts were evaporated to dryness under vacuum, using a rotary evaporator. Total phenolic compounds were measured in accordance with the method of Moongngarm and Setung. [19]. The determination of total phenolic content was performed as gallic acid equivalents (mg/100g), by using the Folin-Ciocalteau phenol reagent and this followed the method described by Iqbal et al. [20]. The diluted methanol extracts (0.2 mL) were added, with 0.8 mL Folin-Ciocalteau phenol reagent and 2.0 mL of sodium carbonate (7.5%), in the given order. The mixtures were vigorously vortex-mixed and diluted to 7 mL of deionized water. The reaction was allowed to complete for 2 h in the dark, at room temperature, prior to being centrifuged for 5 min at 1,259 g. The supernatant was measured at 765 nm, on a Shimazu spectrophotometer. Methanol was applied, as a control, by replacing the sample. Gallic acid was used as a standard and the results were calculated as gallic acid equivalents (mg/g) of the sample. The reaction was conducted in triplicate and the results were averaged. The total flavonoid content was analyzed by the method of Moreno et al. [21]. Respective extracted samples (0.5 mL) were mixed with 0.1 mL of 10% Aluminum nitrate and 0.1 mL of 1 M Potassium acetate and the 4 mL of Ethyl alcohol (80%), and the reaction was allowed to complete for 40 min at room temperature. Then the absorption was measured by the spectrophotometer (Optizen 3220 UV, Mecasys, Daejeon, Korea) at 415 nm. Quercetin(QE) was used as a standard and the results were calculated as QE equivalents (mg/g) of the sample.

2.2.9. Antioxidant capacity analysis

The antioxidant capacity of the grain extracts was evaluated by their free radical-scavenging activity on the DPPH method described by Blois *et al* [22] with a minor modification. Different aliquots of extract solutions prepared by adding 20 mg of methanol extract and 10 mL of methanol was mixed with 2 mL of methanol solution containing the DPPH radical (0.2m M). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a methanol blank without DPPH. The radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = [(Abs_{DPPH} - Abs_{Sample})/Abs_{DPPH}] \times 100$$

Abs_{DPPH}: the absorbance of the DPPH solution without extract

Abs_{Sample}: the absorbance of the sample solution

Also, the antioxidant capacity of the grain extracts was evaluated by the reduction of the $ABTS^+$ radical method as described by Re *et al* [23]. $ABTS^+$ was produced by reacting 7 mM ABTS aqueous solution with 2.45 mM potassium persulfate in the dark for 12-16 h at room temperature. This radical form remains stable for more than 2 days at room temperature. Freshly prepared $ABTS^+$ solution was diluted with 0.01 M phosphate buffered saline (PBS, pH 7.4), and absorbance was adjusted to 0.70 at 734 nm. 0.2 mL of various concentrations of the sample and ascorbic acid standard solution were mixed with 0.8 mL of $ABTS^+$ solution. Finally, the absorbance was measured at 734 m against a blank after a 5 min reaction time at room temperature. The controls contained the extraction solvent instead of the test sample. The scavenging activity of ABTS free radicals was calculated as follows:

$$\text{ABTS radical scavenging activity (\%)} = [(Abs_{control} - Abs_{Sample}) / Abs_{control}] \times 100$$

$Abs_{control}$: the absorbance of the control solution without extract

Abs_{Sample}: the absorbance of the sample solution

2.2.10. Statistical Analysis

All measurements were repeated 3 times and statistical analysis was performed using SPSS (version 18.0, package for Social Science, Chicago, IL, USA). The statistical significance test was conducted by the ANOVA test, and when there were statistically

3. Results

significant differences, the post-hoc test was performed by the Duncan's multiple test. The significance level used for rejection of the null hypothesis was 5% ($p<0.05$).

3.1. α-Amylase Activity

Table 1 shows the α-Amylase activities of quinoa, amaranth and brown rice. The α-Amylase activity was significantly different according to the germination period of grains (p=0.000, respectively). The α-amylase activity of quinoa was the lowest in the 6 h germination group and increased in proportion to the germination period until 24 h and decreased again in the 48 h germination group (p=0.000). The α-Amylase activity of amaranth showed a similar trend to that of quinoa, but it was highest in the 48 h germination group (p=0.000). The α-Amylase activities of quinoa and amaranth were higher than that of germinated barley malt, that was 19.8±0.83 Unit. On the other hand, brown rice showed the lowest α-Amylase activity, and in terms of the germination period, the α-Amylase activity of brown rice was significantly higher in the 48 h germination group. Similar trends of α-amylase activity of germinated brown rice were observed in a previous study [19]. Results showed that a steady increase with advancement of the germination time for up to 72 h germination time and subsequently it reached the maximum value of 21.0 and 42.5 U/g DM after 96 h germination time in brown rice. A number of factors including cultivar and growing location can affect amylase activity with a large variation [19]. A high α-amylase activity of quinoa was reported earlier. Mechanical abrasion of quinoa seed about 18 % of kernel weight increased α-amylase activity in the previous study [24]. Among seven grains including buckwheat, barley, oat, wheat, sorghum, rye and brown rice, germinated barley (approximately 35%) and sorghum (approximately 23%) were the most potent inhibitors of α-amylase activity according to the Kim *et al* [10] and Donkor *et al* [13]. The inhibition of α-amylase activity is considered to be an effective strategy for the control of diabetes by decreasing the absorption of glucose [24,25]. Therefore, the present results suggested that the germinated quinoa and amaranth can be used as potent modulators against the postprandial glucose increase caused by starch hydrolysis.

3.2. Protease Activity

Table 1 shows protease activity of quinoa, amaranth and brown rice. Protease activity increased in proportion with the germination period. Both quinoa and amaranth showed the lowest protease activity in the 6 h-germination group, and the protease activity of them increased in proportion with germination time, and was significantly higher in the 48 h-germination group. As for germinated brown rice, it showed the lowest protease activity of all grains, and although there was no significant difference in protease activity up to 24 h, the highest protease activity was observed in 48 h-germination group ($P<0.05$). The protease activity of 48 h-germinated Andean cereals was about 1.7 times higher than that of barley malt (31.76±0.29 Unit).

3.3 Lipase Activity

Although the lipase activity were tended to increase as the germination period, all samples' lipase activity were not changed significantly (Table 1) The averages lipase activity of GQ and GB were a little higher than the control barley malt (1.38±0.35 Unit).

3.4. GABA content

Table 2 shows the GABA content of quinoa, amaranth and brown rice. The GABA content was significantly different according to the germination period of grains. The GABA content of quinoa was the lowest in the 6 h germination group and increased in proportion to the germination period until 48 h (p= 0.033). The GABA content of amaranth showed dramatic increase by the germination time, which was highest in the 48 h germination group as 8 folds of 6 h germination group (p=0.004). On the other hand, brown rice showed the lowest GABA content, and in terms of the germination period, it was significantly decreased during germination. Oh [26] reported significant increases in the concentration of GABA in brown rice during germination. Donkor et al. [13] observed GABA contents of all seven grains were increased after germination. Specifically, the increase rate was higher in rye, barley, and sorghum compared with other grains. GABA is a non-protein amino acid that is primarily produced from the γ-decarboxylation of L-glutamic acid that is catalyzed by the enzyme, glutamate decarboxylase [13]. GABA acts as a major inhibitory neurotransmitter *in vivo* [13]. Previous results showed that plant extract containing high levels of GABA are effective in blood pressure regulation and in the recovery of alcohol related symptoms.

This shows that there is potential for producing GABA-enriched food resources with germinated quinoa and amaranth.

Table 2. GABA content of test cereals with various the germination time

(mg/100g)germination time (h)	†G-Quinoa		G-Amaranth		G-Brown rice	
6	12.01	(0.79)[d]	9.92	(0.08)[d]	6.85	(0.01)[a]
12	19.93	(1.10)[c]	47.14	(0.18)[b]	4.00	(0.31)[b]
24	28.85	(0.27)[b]	21.02	(1.96)[c]	2.52	(0.14)[c]
48	31.23	(1.85)[a]	80.87	(9.87)[a]	2.48	(0.10)[c]
P-value	0.033		0.004		0.006	

†Germinated

§ mean (SD) with different letters indicate significant difference ($p<0.05$)

Table 1. α-Amylase , protease and Lipase activity of test cereals with various germination time

α-Amylase activity				Protease activity						Lipase activity					
G-Amaranth		G-Brown rice		†G-Quinoa		G-Amaranth		G-Brown rice		†G-Quinoa		G-Amaranth		G-Brown rice	
24.88	(0.45[a])	3.80	(0.42[a])	§§41.58	(0.96[a])	36.76	(0.47[a])	18.38	(0.24[a])	§§§1.43	(0.28)	0.74	(0.45[a])	3.80	(0.42[a])
29.34	(0.39[b])	3.68	(0.24[a])	47.02	(1.13[b])	43.80	(1.70[b])	18.18	(0.86[a])	1.45	(0.98)	0.69	(0.00)	1.68	(0.85)
30.79	(0.51[c])	3.68	(0.23[a])	53.39	(0.52[c])	47.00	(1.43[c])	18.18	(0.15[a])	1.57	(1.06)	0.68	(0.61)	1.68	(0.31)
37.11	(0.05[d])	5.05	(0.31[b])	54.01	(0.20[c])	55.13	(1.78[d])	20.42	(1.16[b])	2.16	(0.50)	1.06	(0.55)	1.69	(0.66)
0.000		0.000		0.000		0.000		0.001		0.214		0.352		0.973	

†Germinated,
§ mean(SD) of Unit (1 unit : 0.01 increase O.D. per10 min) with different letters indicate significant difference($p<0.05$)

Germination Time(h)	†G-Quinoa	
6	§29.28	(1.07^{a})
12	32.98	(0.50^{b})
24	34.39	(0.54^{c})
48	32.71	(1.20^{b})
P-value	0.000	

3.5. Vitamin content

Vitamins are the essential micronutrients that are required as vital compounds in the human diet [24]. Andean pseudocereals possessed desirable composition of vitamins and minerals when compared to wheat, oats and rice [24,27]. Table 3 shows the thiamin contents of quinoa, amaranth and brown rice. The thiamin contents of quinoa and brown rice were significantly decreased according to the germination period (p=0.013, 0.000, respectively). The thiamin content of quinoa was the lowest in the 48 h germination group. The thiamin content of amaranth showed a similar trend to that of quinoa, but it was not significant. The thiamin content of 6 h-germinated quinoa and brown rice was similar to that of germinated barley malt (2.13±0 mg/100g). On the other hand, amaranth showed the lower than those of others. Muyonga et al.[28] reported that raw amaranth seeds contain 0.1 mg/100g of thiamin.

As shown in Table 3, niacin content of quinoa (P=0.000), amaranth(P=0.000) and brown rice (P=0.006) was significantly different according to the germination period. The niacin content of quinoa was the lowest in the 6 h germination group and it was significantly increased as the germination time until 48 h up to two fold of 6 h. Similar patterns in the content of niacin of both amaranth and brown rice were observed. There were not enough comparable studies on the germination-related nutrition values changes for the quinoa and amaranth. Colmenares de Ruiz & Bressani [9] observed that thiamin contents of two amaranth species (*A. cruentus and A. hypochondriacus*) significantly increased but one (*A.caudatus*) did not among three amaranth spaecies. There was no significant difference in niacin content between species and among germination time. Monngngarm & Setung reported that the germination of white rice brought about a significant reduction of thiamine content (-47.8%) compared to that of ungerminated rice. Results may be due to the effect of soaking the dehulled rice seed and changing the water, which led to a leaching out of water soluble vitamins, whereas there was no significant effect of soaking and water changing on the B vitamins in the brown rice, because it was protected by the hull. Also, they found that the level of thiamin was slightly increased, but not significantly. Similar but significant changes were reported in the niacin content after germination in brown rice, which are in the accordance with the present study.

Table 3 shows the ascorbic acid contents of quinoa, amaranth and brown rice. The ascorbic acid contents of brown rice and germinated barley malt were fall short of the level of quantification. On the other hand, germinated quinoa and amaranth had high level of ascorbic acid. The ascorbic acid content of germinated quinoa was specifically the highest among test samples. The ascorbic acid content of quinoa was the lowest in the 6 h germination group and increased in proportion to the germination period until 48 h (p =0.000), although it was not significantly different between 24 h and 48 h germination. Similarly, the ascorbic acid content of amaranth was the lowest in the 6 h germination group and increased in proportion to the germination period until 48 h (p =0.000). Similar results were documented in significant increase of ascorbic acid content after germination of amaranth by Chen & Thacker [9,29]. Amaranth was considered to be a rich source of ascorbic acid and the content of vitamin E were higher in the seeds of quinoa [24], which were not accordance with the present results. However, Svirskis [30] observed 4.2 mg/100g of ascorbic acid, which could be comparable to the present results.

3.6. Total polyphenol contents

Phenolic compounds are classified under the group of bioactive compounds possessing strong antioxidant activity [24]. Table 4 shows the total polyphenol contents (TPC) of quinoa, amaranth and brown rice. Significant differences were observed for TPC, amongst the samples. The TPC of germinated quinoa was the highest among test samples. The TPC of quinoa was the lowest in the 6 h germination group and increased in proportion to the germination period until 48 h (p =0.011). Also, the TPC content of amaranth was the lowest in the 6 h germination group and increased in proportion to the germination period until 48 h (p =0.042). The levels of TPC in both quinoa and amaranth, that were produced in order to adapt to the climatic conditions of central-western Brazil, reported, quinoa had higher concentration of TPC than amaranth such as 0.63(3.88) mg/g for quinoa and 0.22(5.23) mg/g for amaranth. Furthermore, the phenolic compounds of those grains were regarded as the sources of antioxidant activity based on a good correlation between TPC and antioxidant capacity (r=0.871) [1]. Among the pseudocereals, quinoa had higher content of TPC when compared to that the seed of amaranth and kaniwa [31]. The content of TPC in quinoa and amaranth were similar to that in sorghum, whereas those were relatively lower compared to cereals like oats, rye and barley [24].

In the present study, the TPCs of brown rice was not significantly different by the germination time, ranged from 1.25-1.33 mg /g. Iqbal *et al.* [20] reported that TPC was in the rage of 2.51-3.59 mg /g of the rice bran extracts. On the other hand, Moongngarm & Setung [19] observed 0.70 mg/g of TPC in ungerminated brown rice and 0.84 mg /g in germinated brown rice. Since the concentration of TPC was high in the bran layer [19], the way of sample extracts and other factors, such as cultivar and growing location may affect the concentration of TPC. Moreover, the change of TPC was also rely on the type of phenolic compounds, which dominated in the different rice cultivar. 6-*O*-feruloylsucrose and 6-*O*-sinapolysucrose were the major soluble phenolic compounds, in brown rice and there were significant decreases during germination for 24 h, whereas the levels of free ferulic acid and sinapinic acid increased significantly [19].

3.7. Flavonoid contents

Flavonoids are the common class of phenolic compounds which attributes to antioxidant and lipid reducing properties [24, 32]. The pseudocereals also contained caffeic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid is widely present in cell wall of plants that possess antioxidant activities in food substances. The arrangement of aromatic rings determines the extent of antioxidant activity in the structure of ferulic acid and its derivatives. The content of p-coumaric acid and vanillic acid was higher in the seeds of quinoa [24,31]. In Table 4, the total flavonoid contents of quinoa, amaranth and brown rice were increased in proportion to the germination period. The total flavonoid was the highest concentration in quinoa compared with those in other samples. Apparently there is not much information about antioxidant activities of quinoa and amaranth after germination.

3.8. Antioxidant activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [33]. The darkcolor of the DPPH radical solution becomes lighter when it is mixed with an antioxidant. It is widely used to evaluate the antioxidant activity of various compounds. Table 5 shows the DPPH of quinoa, amaranth and brown rice. Germinated quinoa extract showed the highest DPPH radical scavenging activity among test sample grains, which was ranged from 88.20-91.42%. The DPPH radical scavenging activity of quinoa was the lowest in the 6 h germination group and tended to increase in proportion to the germination period until 24 h but it was decrease in 48 h germination group with the similar level of the 6 h germination group. The DPPH radical scavenging activity of amaranth was significantly increased as the germination period until 48 h (p =0.031). On the other hand, the DPPH radical scavenging activity of brown rice was not significantly changed by the germination period but it was quite high from 75.35 to 75.79%.

Table 3. Thiamin, niacin and ascorbic acid content of test cereals with various germination time

Niacin content			Ascorbic acid content		
†G-Quinoa	G-Amaranth	G-Brown rice	†G-Quinoa	G-Amaranth	G-Brown rice
0.32 (0.03)c	0.31 (0.03)d	0.23 (0.00)c	21.89 (0.18)c	3.50 (0.21)d	nd
0.39 (0.01)c	0.43 (0.02)c	0.34 (0.03)ab	28.12 (0.19)b	6.09 (0.01)c	nd
0.60 (0.03)b	0.70 (0.01)b	0.41 (0.02)b	35.91 (0.30)a	7.44 (0.29)b	nd
0.66 (0.02)a	0.80 (0.02)a	0.59 (0.08)a	37.68 (1.36)a	8.39 (0.12)a	nd
0.000	0.000	0.006	0.000	0.000	

†Germinated, mg/100g
$ mean(SD)with different letters indicate significant difference(p<0.05)

Germination time (h)	Thiamine content					
	†G-Quinoa		G-Amaranth		G-Brown rice	
6	2.75	(0.26)^{a}	0.13	(0.04)	2.14	(0.04)^{a}
12	2.60	(0.13)^{a}	0.13	(0.02)	1.71	(0.04)^{b}
24	2.12	(0.05)^{b}	0.14	(0.02)	1.39	(0.02)^{c}
48	1.88	(0.05)^{b}	0.12	(0.01)	1.02	(0.01)^{d}
P-value	0.013		0.922		0.000	

In a previous study, the lowest concentration necessary for 50% inhibition of DPPH was obtained in quinoa as 313.25 µg/ml, that was the half of amaranth as 638.67µg /ml with ungerminated grains [1]. The TPC and DPPH results in germinated quinoa in the present study seemed to be similar to the levels of germinated rye in the previous results of Donkor et al although the germination conditions including germination temperature and time were somewhat different.
The ABTS radical cation decolorization test is another method that is widely used to evaluate antioxidant activity. The reaction between ABTS and potassium persulfate generates ABTS radicals. When mixed with an antioxidant, an electron is donated to the ABTS radical, which converts it to a non-radical form. Decolorization indicates a reduction in the ABTS radical concentration. All grain sample extracts in this study had significant ABTS radical scavenging activity over 92% as shown in Table 5. Germinated quinoa extract showed the highest ABTS radical scavenging activity, which was ranged from 87.22-99.41%. The ABTS radical scavenging activity of quinoa was the highest in the 6 h or 12h germination group and decreased in proportion to the germination period until 48 h (p=0.027). On the other hand, the ABTS radical scavenging activity of amaranth was significantly increased in proportion to the germination period until 48 h up to 98.99% (p =0.040). The ABTS radical scavenging activity of brown rice was significantly decreased in proportion to the germination period until 48 h. The ABTS radical scavenging activity of brown rice was the highest in the 12 h-germination group.
Recent studies have suggested that bioactive compounds, especially polyphenols help in reducing the risk of neurodegenerative and diabetic diseases and regulation of apoptosis in tumor cells [24,34]. The seeds of amaranth, quinoa as well as brown rice possessed higher content of bioactive compounds such as phenolic compounds and flavonoids when compared to legumes [24,31].
Moreover, these capacity was observed to increase during the germination in the present study.

4. Conclusion

This study was conducted to compare the changes of α-Amylase and protease activity by using water-soluble extracts of germinated samples after germination of quinoa, amaranth and brown rice for 6 to 48 h under the same conditions. As for quinoa and amaranth, α-Amylase and Protease activities were significantly increased in proportion to the germination period until 48 h-germination, and their enzyme activities were significantly higher than that of germinated brown rice or malt. Considering the starch content and proteolytic power of quinoa and amaranth, they are considered to be very effective and efficient materials that can be applied to various food and biotech industries. This study focused on the changes in enzyme activity according to germination time, but since changes in nutritional and functional components may be affected by various germination conditions such as the germination temperature and soaking solution, there is a need to conduct further research for optimization of such various germination conditions. However, this study confirmed the potential utilization values of quinoa and amaranth by providing the analysis results of their enzymatic activities according to the germination period for which there have not been sufficient domestic research data available to date, and the study results of this study warrant future studies on high value-added food development and industrial application using them.

Table 4. Total polyphenol content and Total flavonoid content of test cereals with various the germination time

(mg/g)Germination Time(h)	Total polyphenol content						Flavonoids content					
	†G-Quinoa		G-Amaranth		G-Brown rice		G-Quinoa		G-Amaranth		G-Brown rice	
6	2.39	(0.00)^{d}	1.47	(0.02)	1.25	(0.00)	0.52	(0.00)^{d}	0.22	(0.00)	0.09	(0.00)^{c}
12	2.74	(0.01)^{c}	1.80	(0.02)^{b}	1.33	(0.00)	0.64	(0.00)^{c}	0.22	(0.01)	0.09	(0.00)^{c}
24	3.11	(0.01)^{b}	1.78	(0.01)^{b}	1.26	(0.01)	0.78	(0.02)^{b}	0.27	(0.00)	0.15	(0.00)^{b}
48	3.33	(0.01)^{a}	2.44	(0.00)^{a}	1.29	(0.00)	1.08	(0.04)^{a}	0.27	(0.00)	0.25	(0.00)^{a}
P-value	0.011		0.042		0.542		0.021		0.502		0.034	

†Germinated
§ mean(SD) with different letters indicate significant difference($p<0.05$)

Table 5. DPPH and ABTS raical scavenging activities of test cereals with various the germination time

Germination Time(h)	DPPH raical scavenging activity (%)						ABTS raical scavenging activity (%)					
	[†]G-Quinoa		G-Amaranth		G-Brown rice		G-Quinoa		G-Amaranth		G-Brown rice	
6	88.20	(0.62)	29.01	(0.00) [c]	75.52	(0.08)	99.15	(0.09) [a]	92.65	(0.42) [c]	98.78	(1.06) [ab]
12	90.00	(0.08)	39.69	(0.15) [b]	75.68	(0.00)	99.41	(0.09) [a]	95.79	(0.40) [b]	99.89	(0.09) [a]
24	91.42	(0.08)	34.84	(0.08) [b]	75.79	(0.00)	96.33	(0.00) [b]	95.90	(0.24) [b]	96.27	(1.48) [b]
48	88.47	(0.54)	61.45	(0.39) [a]	75.35	(0.15)	87.22	(0.58) [c]	98.99	(0.09) [a]	93.61	(1.21) [c]
P-value	0.845		0.031		0.542		0.027		0.040		0.082	

[†]Germinated
[§] mean(SD) with different letters indicate significant difference(p<0.05)

Acknowledgement

The research for this study was conducted with the financial support of the research project grant (PJ0113582017, PJ0113542017 & PJ01349201) from the Rural Development Administration.

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