Enhancement of the Anti-hyperglycemic and Antioxidant Activities of Wheat by the Germination Process

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Inhibition of α-glucosidasesinvolved in the digestion and absorption of carbohydrates can decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet. Therefore, we investigated the changes in α-glucosidase inhibitory activity of wheat during germination process. We also investigated total phenolics and oxygen radical absorbance capacity (ORAC) during germination process of wheat. Wheat was germinated for 0, 12, 24 and 48 hrs and results showed that both phenolic contents and α-glucosidase inhibitory activities increased with germination time. More specifically, total phenolic content increased from (293mg/100g-F.W.) at 0 hrs germination to a high (4,082mg/100g-F.W.) achieved after 48 hrs germination. Additionally, alpha-glucosidase inhibitory activity was dramatically increased from a low 10% inhibition at 0 hrs germination to a high 60% inhibitory activity observed at 48 hrs. These results suggest that wheat, depending on the germination time, has the potential to contribute as a dietary supplement for controlling hyperglycemia and oxidative stress-linked diabetes complications. It may due to the natural response of plant seed to overcome the biotic/abiotic stress from environment during germination period.

Ⅰ. Materials and Methods

1) Metarials

Wheat was purchased from local market (Daejeon, Korea). Twenty fivemale Sprague-Dawley (SD) rats, aged 5 weeks, were purchased from Central Lab. Animal Inc (Seoul, Korea). All reagents used in this experiment were all from Sigma Chemical Co. (St. Louis, MO, U.S.A.) preparation of extracts

2) Germination and sample preparation

After overnight soaking of wheat seeds in dark room, germination was performed for 0, 12, 24, and 48 hours at room temperature (25℃). After drying 10 g of germinated seed was mashed and stirred respectively in

100 mL of distilled water at 100℃ for 30 min. The germinated wheat(GWE) seed extract and non-germinated wheat extract (NWE) were then filtered through a Whatman # 2 filter, centrifuged at 7,000 × g for 1 hr, vacuum-evaporated at 45℃, freeze-dried and kept at -70℃ until analysis.

Table 1. Comparison of the yield of solid content with different germination times of wheat.

	Yield (%)
0 hr	1.67 ± 0.43
12 hr	3.55 ± 0.05
24 hr	12.35 ± 1.69
48 hr	25.68 ± 0.61

3) α-Glucosidase Inhibition Assay

Rat intestinal α-glucosidase assay referred to the method of Kwon *et al.* with slight modification. A total of 1 g of rat-intestinal acetone powder was suspended in 3 mL of 0.9% saline, and the suspension was sonicated 12 times for 30 sec at 4℃. After centrifugation (10,000 g, 30 min, 4℃), the resulting supernatant was used for the assay. Sample solution (50 μL) and 0.1 M phosphate buffer (pH 6.9, 100 μL) containing glucosidase solution (1.0 U/mL) was incubated at 25℃ for 10 min. After pre-incubation, 5 mMp-nitrophenyl-ⷭ-D-glucopyranoside solution (50 μL) in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25℃ for 5 min. Before and after incubation, absorbance was read at 405 nm and compared to a control which had 50 μL of buffer solution in place of the extract by micro-plate reader (SUNRISE; Tecan Trading AG, Saltzburg, Austria). The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

(A)

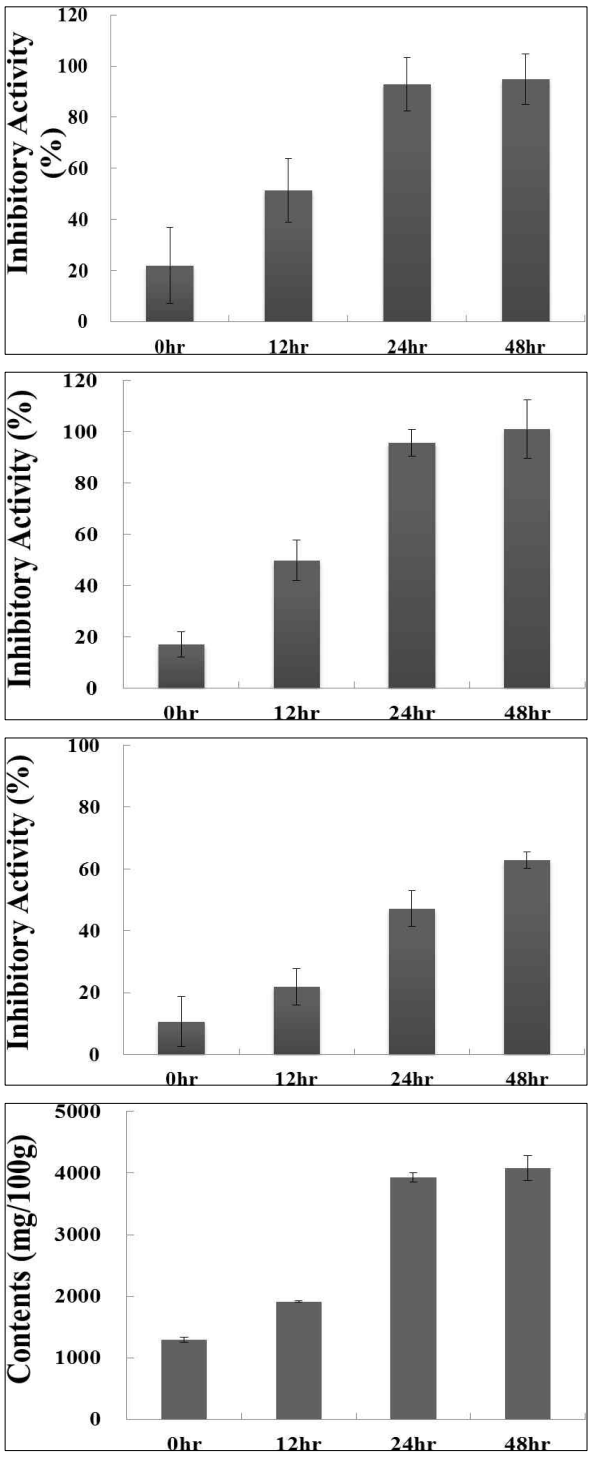

Fig. 1. Dose dependent changes in rat intestinal α-glucosidase (A),maltase (B), and glucoamylase inhibitory activities (C) with different germination times of wheat.

$$\% \text{ inhibition} = \left(\left[\frac{\Delta A_{405}^{Control} - \Delta A_{405}^{Extract}}{\left[\Delta A_{405}^{Control} \right]} \right] \right) x100$$

4) Maltase and Sucrase Inhibition Assay

The crude enzyme solution prepared from rat intestinal acetone powder Sigma-Aldrich Co. (St. Louis, MO, USA) was used as the small intestinal maltase and sucrase, showing specific activities of 0.70 and 0.34 units/mL, respectively. Rat-intestinal acetone powder (1.0 g) was suspended in 3 mL of 0.9% saline, and the suspension was sonicated twelve times for 30 sec at 4℃. After centrifugation (10000 X g, 30 min, 4℃), the resulting supernatant was used for the assay. Maltase and sucrase inhibitory activities were assayed by modifying a method developed by Dahlqvist. The inhibitory activity was determined by incubating a solution of an enzyme (50 mL), 0.1 M phosphate buffer (pH 7.0, 100 mL) containing 0.4 mg/mL sucrose or maltose, and a solution (50 mL) with various concentrations of sample solution (between 0.05 mM and 1.0 mM) at 37℃ for 30 min. The reaction mixture was heated in a boiling water bath to stop the reaction for 10 min, and then the amount of liberated glucose was measured by the glucose oxidase method. The inhibitory activity was calculated from the formula as follows. Inhibition (%) = (C-T)/C x 100, where C is the enzyme activity without inhibitor and T is the enzyme activity with inhibitor.

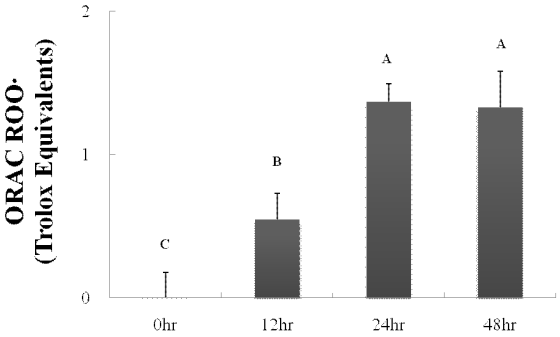

Fig. 3. Changes in ORAC values of germinated wheat by germination time

5) Sugar and Starch Loading Test

Effect on hyperglycemia induced by carbohydrate

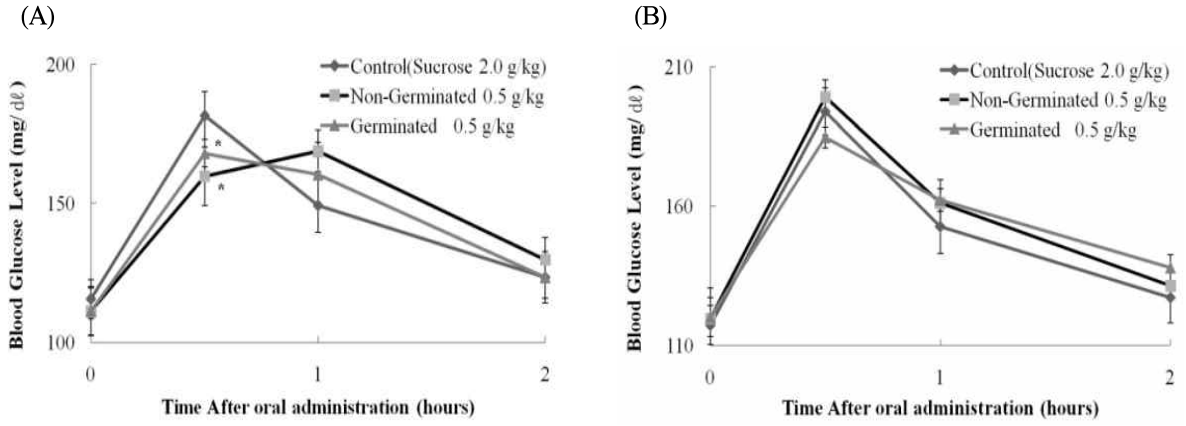

Fig. 2. Effect of germination of wheat on blood glucose level using sucrose(A) and starch(B) loading test.

After fasting for 24 hours, 6-week-old, male SD rats were orally administered with sucrose solution(2.0 g/kg) with or without samples. Each point represents mean±S.D.(n=5). $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ compared to different samples at the same concentration by unpaired student's t-test.

loads in Sprague-Dawley (SD) rats were determined by the inhibitory action of germinated wheat, non-germinated wheat, and Acarbose on postprandial hyperglycemia. Five week-old male SD rats were purchased from Joongang Experimental Animal Co. (Seoul, Korea) and fed a solid diet (Samyang Diet Co., Seoul, Korea) for one week. The rats were housed in a ventilated room at 25 ± 2℃ with 50 ±7% relative humidity, and under an alternating 12 hour light/dark cycle. After 5 groups of 5 male SD rats (180~200 g) were fasted for 24 h, 2.0 g/kg of sucrose and starch were orally administrated concurrently with 0~500 mg/kg inhibitors (germinated wheat, non-germinated wheat and acarbose). The blood samples were then taken from the tail after administration and blood glucose levels were measured at 0, 0.5, 1, 2 and 3 hours. The glucose level in blood was determined by glucose oxidase method and compared with that of the control group, which had not taken the inhibitors. The parameters for blood glucose levels were calculated using WinNonLin program (Version 5.2.1, Pharsight Corporation, Cary, NC, USA). Maximum observed peak blood glucose level (C_{max}) and the time at which it is observed (T_{max}) were determined based on the observed data. Area under the blood glucose-time curve up to the last sampled time-point (AUC_{last}) was estimated by the trapezoidal rule.

6) Oxygen radical absorbance capacity (ORAC) assay

Antioxidant activities of germinated wheat extracts in different concentrations(between 10 and 100 mg/ml) were investigated for their peroxyl and hydroxyl radical-scavenging capacities using ORAC assay system. The ORAC assay was carried out using a TecanGENios multi-functional plate reader (GENios Tecan Trading AG, Salzburg, Austria) with fluorescent filters (excitation wavelength: 485 nm, emission filter: 535 nm). In the final assay mixture, fluorescein (40 nM) was used as a target of free radical attack with either 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH, 20 mM) as a peroxyl radical generator in peroxyl radical-scavenging capacity (ORACROO•) assay [22] or with H_2O_2-$CuSO_4$ (H_2O_2, 0.75%; $CuSO_4$, 5mM) as a hydroxyl radical generator in hydroxyl radical-scavenging capacity (ORACHO•) assay [15]. Trolox (1 mM) was used as a control standard and prepared fresh on a daily basis. The analyzer was programmed to record the fluorescence of fluorescein every 2 min after AAPH or H_2O_2 - $CuSO_4$ was added. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and each sample. All data were expressed as micromoles of Trolox equivalents (TE). One ORAC unit is equivalent to the net protection area provided by 1 mM of Trolox.

7) Total flavonoid assay

Total flavonoid was measured by using the method

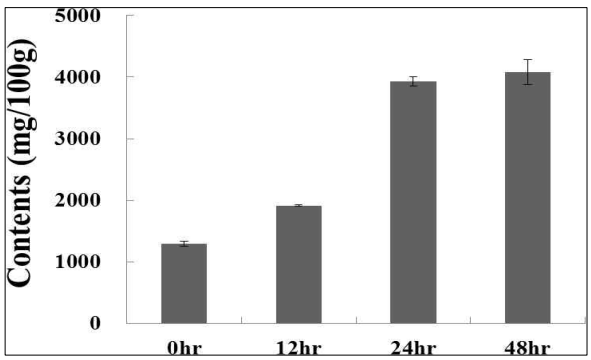

Fig. 4. Changes in total phenolic contents of germinated wheat by germination time

of Moreno et al.. A volume of 0.1 mL of sample extract solution was added 0.9 mL of 80% ethanol. Half mL of mixture solution was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 80% ethanol, and allowed to stand for 40 min at room temperature. The absorbance was read at 415 nm using spectrophotometer (Shimadzu Inc., Japan). Standard curve was established using various concentrations of quercetin. Absorbance values were converted to total flavonoids which were expressed as milligrams of quercetin equivalent per gram of sample extract.

Ⅱ. Discussion

1. Germinated wheat extracts had high α-glucosidase inhibitory activity.(Fig. 1)
2. Germination time effects on the α-glucosidase inhibitory activity, antioxidant potential and total phenolic contents(Fig. 1)
3. Carbohydrate hydrolyzing enzyme inhibition was enhanced by germination time and germinated wheat had higher reduced postprandial increase of blood glucose than non-germinated wheat in-*vivo*(Fig. 2,3,4,)
4. These results indicate that COS may have anti-diabetic effect by suppressing carbohydreate absorption from intestine, and thereby reducing postprandial increase of blood glucose.(Fig. 2,3,4),
5. Germinated wheat extract treatment significantly reduced the fasting glucose level, (Table 1).
6. These results indicate that germination process exerts an anti-diabetic effect through a mechanism that may involve up-regulation of synthesis of phenolic phytochemicals due to the response on biotic and abiotic stress.(Fig. 7,8)
7. These results indicate that germinated wheat extracts may have anti-diabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the postprandial increase of blood glucose.