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# Antioxidant, α-Glucosidase Inhibitory, and Anti-Inflammatory Activities and Cell Toxicity of Waxy and Normal Wheat Sprouts at Various Germination Time

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**Abstract:** Germination is an effective process to improve the bioactivities including antioxidant and hypoglycemic activities of grains, but its effect on waxy wheat has not yet been actively studied. This study, therefore, examined the effect of germination time on the activities of Korean waxy and normal wheat sprouts. The total phenolic and flavonoid contents, and antioxidant activities of the waxy and normal wheat sprouts increased with germination time. Flavonoid content and antioxidant activity were higher in waxy wheat sprouts than in normal ones, but the total phenolic content and α-glucosidase inhibitory activity were not significantly different. The NO production ratio of MEF cells was higher for waxy wheat sprout than for normal ones, thereby indicating lower anti-inflammatory activity of waxy wheat sprouts. The viabilities of Caco-2 cells treated with waxy wheat sprouts was higher than that of cells treated with normal ones for the water extract. These results imply that waxy wheat sprouts exhibit better antioxidant activity and less cell toxicity for water extract, and therefore, could be used as a health-promoting food.

**Keywords:** waxy wheat sprout; germination; antioxidant activity; α-glucosidase inhibitory activity; anti-inflammatory activity; cell toxicity



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## 1. Introduction

Consumers preference towards functional foods is increasing, and these functional foods exert diverse health benefits including antioxidant, hypoglycemic, and anti-inflammatory activities. The processing methods to improve the health benefits of food materials are also preferred, and this includes germination. Germination is a traditional method of processing the grains, which improves the quality characteristics as well as reduces anti-nutritional factors of grains [1,2].

Wheat is widely consumed and easily available, and a variety of products have been developed from wheat grains. Wheat can be classified into two categories, waxy and normal, based on the ratio of amylose and amylopectin contents in starch. Waxy wheat contains a lower amylose content of 0–4% than normal (wild-type) wheat (21–28%) [3,4], in contrast to high amylopectin content in waxy wheat. Low amylose content in wheat is attributed to the mutation in genes encoding the granule bound starch synthase enzyme, which is responsible for amylose synthesis [3,5]. The amylose content in starch impacts the physicochemical properties of wheat flour and the quality of its products [6]. Waxy wheat is known to produce strong gluten in wheat flour [7] and exhibits a shorter dough development time [8].

Germination increases the nutritional components, including folate, dietary fiber, and proteins and enzymatic activities of protease and amylase [9]. Wheat sprout, the germinating product of wheat, has also been reported to possess antioxidant [10], anticancer [11], anti-inflammatory [12], and antibacterial activities [13]. Feeding of wheat sprout to BALB/c male mice treated with streptozotocin to induce diabetes mellitus reduced blood sugar

level, and the reduction in sugar level was positively correlated with the concentration of wheat sprout in the feed [14].

Although many studies have reported the health benefits associated with wheat sprouts, reports on waxy wheat sprouts are limited. As mentioned previously, the effect of waxy wheat on the rheological and technological properties of its product has been reported in several studies, but the bioactivities and changes in the activities during their germination have been rarely studied. Therefore, this study examined various bioactivities, including antioxidant and α-glucosidase inhibitory activities, and the effect on cell viability and nitric oxide (NO) production in Korean waxy wheat sprout (WS) and normal wheat sprout (NS) grown for different germination time periods to elucidate the effect of the time on the activities of these wheat sprouts and compare the activities of WS with NS.

## 2. Materials and Methods

### 2.1. Materials

Korean waxy (Chalmil) and normal (Keumgangmil) wheat seeds (*Triticum aestivum* L.), were obtained from the Korean Wheat Agriculture located in Gwangju, Korea. All wheat seeds were cleaned using distilled water. The seeds were germinated at 20 ± 5 °C for 11 d on soft agar (1.5% agar) and harvested at 7, 9, and 11d. The harvested wheat sprouts were freeze-dried and extracted with water and 80% ethanol. Each sprout (10 g) was stirred with 30 mL of either water or 80% ethanol for 3 h and filtered at ambient temperature. This procedure was repeated thrice. The collected extracts were concentrated to 30 mg/mL and stored at −80 °C. The extracts were used for measurement of total phenolic and flavonoid contents, antioxidant, α-glucosidase inhibitory, anti-inflammatory activities, and cell toxicity of the waxy and normal wheat sprouts.

### 2.2. Cell Culture

Human intestinal epithelial Caco-2 and mouse embryonic fibroblast (MEF) cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) at 37 °C in a humidified atmosphere supplied with 5% $CO_2$. The medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin-streptomycin solution (P/S, Sigma-Aldrich Co., St. Louis, MO, USA).

### 2.3. Total Phenolic Content

Total phenolic content was assessed using the Folin–Ciocalteu method as described previously [15]. Each wheat sprout extract (0.2 mL) was mixed with 20 mL of 2% $NaNO_3$ (Daejung Chemical & Metals Co. Ltd., Siheung, Korea), and incubated for 2 min. Then, 0.2 mL of 50% Folin-Ciocalteu's reagent (Junsei Co. Ltd., Tokyo, Japan) was added, incubated for 30 min and the absorbance was measured at 750 nm. Quercetin (Sigma-Aldrich Co., St. Louis, MO, USA) was used as a standard, and the total phenolic content of the wheat sprout extracts was expressed as quercetin equivalent (QE).

### 2.4. Flavonoid Content

Each wheat sprout extract (0.2 mL) was mixed with 2 mL of diethylene glycol (Sigma-Aldrich Co., Steinheim, Germany) and 0.2 mL of 1 N NaOH (Daejung Chemical & Metals Co. Ltd., Siheung, Korea), incubated at 37 °C for 1 h and the absorbance of the reaction mixture was measured at 420 nm to determine the flavonoid content [16]. Quercetin was used as a standard, and the flavonoid content of the wheat sprout extracts was expressed as quercetin equivalent (QE).

### 2.5. DPPH Radical Scavenging Activity

2,2-Diphenyl-1-picryhydrazyl (DPPH, Sigma-Aldrich Co., St. Louis, MO, USA) radical scavenging activity was determined using the method described by Blois [17]. A mixture of wheat sprout extract (0.5 mL) and 5 mL of 0.4 mM DPPH in methanol was incubated in

the dark for 30 min at room temperature. The absorbance was measured at 517 nm and the DPPH radical scavenging activity of the extracts was expressed as trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma-Aldrich Co., St. Louis, MO, USA) equivalent antioxidant capacity (TEAC).

### 2.6. ABTS Radical Scavenging Activity

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich Co., St. Louis, MO, USA) radial scavenging activity was determined using the method described by Fellegrini et al. [18] with slight modifications. The radical cation was produced through reacting 7 mM ABTS stock solution with 140 mM potassium persulfate (Sigma-Aldrich Co., Steinheim, Germany) and incubating the mixture in the dark for 16 h until the reaction was complete. The $ABTS^{\cdot+}$ stock solution was diluted in ethanol to an absorbance of 0.7 ± 0.002 at 734 nm. Each wheat sprout extract (150 µL) was mixed with 3 mL of diluted $ABTS^{\cdot+}$ solution, incubated for 3 min, and the absorbance was measured at 734 nm. The ABTS radical scavenging activity of the extracts was determined and expressed as TEAC.

### 2.7. Reducing Power

The mixture containing 0.1 mL of each wheat sprout extract, 0.5 mL of 0.2 M sodium phosphate buffer (pH 6.0), and 50 µL of 1% potassium ferricyanide (Sigma-Aldrich Co., St. Louis, MO, USA) was incubated at 50 °C for 20 min. After adding of 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 650 rpm for 10 min, and the supernatant solution was collected. The solution (0.5 mL) was mixed with 0.5 mL of distilled water and 0.1 mL of 1% ferric chloride (Sigma-Aldrich Co., Steinheim, Germany), and the absorbance was determined at 700 nm [19]. The reducing power of the wheat sprout extracts was expressed as trolox equivalent reducing power (TERP).

### 2.8. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibitory activity was measured using the described by Matsui et al. [20] with modifications. The volume of the mixture of 2.5 mM NaCl, 20 units of α-glucosidase (Sigma-Aldrich Co., St. Louis, MO, USA), and 12.5 mL of 50 mM phosphate buffer (pH 7.0) were adjusted to 25 mL with distilled water to prepare the enzyme solution. Each wheat sprout extract (20 µL) was reacted with 80 µL of enzyme solution at 37 °C for 5 min, and then with 1.9 mL of 0.7 mM *p*-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich Co., St. Louis, MO, USA) prepared in 50 mM phosphate buffer (pH 7.0) at 37 °C for 15 min. The reaction was stopped through adding 2 mL of 0.5 M Tris solution. The absorbance was measured at 400 nm, and the activity was calculated using the following equation.

$$\alpha\text{-Glucosidase inhibitory activity (\%)} = [1 - (A/B)] \times 100$$

where A: absorbance of reactant with each wheat sprout; and B: absorbance of reactant without wheat sprout.

### 2.9. Nitric Oxide Production Ratio

NO production in MEF cells was quantified using the Griess reagent system (Promega Co., Madison, WI, USA). The cultured cells were seeded in 24-well cell culture plates at 5x104 cells/well, pretreated with 10 µL of wheat sprout for 24 h, and then subsequently reacted with a cytokine cocktail (CT: 50 ng/mL TNF-α + 50 ng/mL IFN-γ + 25 ng/mL IL1β + 10 µ/mL LPS) for an additional 24 h. The NO production was measured following to the manufacturer's instructions. The absorbance was measured at 540 nm using the microplate reader. NO production ratio was calculated with CT stimulation compared to the absence of CT stimulation for each wheat sprout.

### *2.10. Cell Viability*

The viability of Caco-2 cells was determined using the WST assay kit (EZ-Cytox, Daeil lap service Co. Ltd., Seoul, Korea). The cultured cells were pretreated using 10 μL of each wheat sprout for 24 h and WST assay was conducted following the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate reader (xMarkTM Microplate Absorbance Spectrophotometer, Bio-Rad Inc., Hercules, CA, USA) after 3 h.

### *2.11. Statistical Analysis*

All measurements in this study were performed at least in duplicate. Data were analyzed statistically using ANOVA and Duncan's multiple range test at $p < 0.05$ with IBM SPSS statistics (ver. 21. IBM Corp., Armonk, NY, USA). Pearson's correlation coefficient between total phenolic and flavonoid contents, and the bioactivities was calculated and a heatmap was generated using GraphPad Prism (ver. 9.2.0. GraphPad, CA, USA).

## 3. Results and Discussion

This study examined the effect of germination time on bioactivities including antioxidant and α-glucosidase inhibitory activities, and the effect on cell viability and NO production of Korean WS and NS. Wheat sprouts were extracted with water or 80% ethanol to determine their activities, and the activities were compared between the two solvents to identify more beneficial extract.

### *3.1. Total Phenolic and Flavonoid Contents*

Total phenolic and flavonoid contents of water and 80% ethanol extracts of WS and NS cultured for 7, 9, and 11 days are exhibited in Figure 1. The total phenolic content of water and 80% ethanol extracts of both wheat sprouts increased with germination time except for the water extract of NS (Figure 1A). The content of the water extract of NS increased until 9 days of germination, but there was no significant difference between the extracts at 9 and 11 days. The total phenolic content of water extracts was higher than that of 80% ethanol extracts in each wheat sprout, but it was not significantly different between WS and NS.

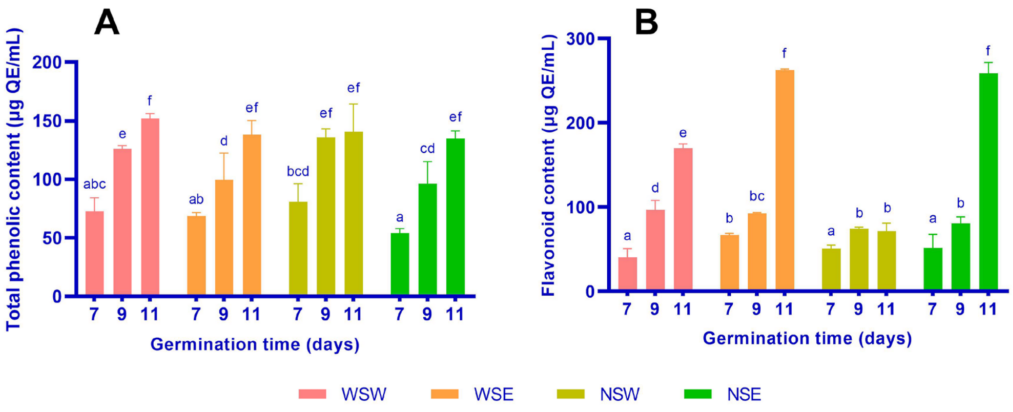


**Figure 1.** Total phenolic (**A**) and flavonoid (**B**) contents of Korean waxy and normal wheat sprouts germinated for 7, 9, and 11 days. WSW, water extracts of the waxy wheat sprouts; WSE, 80% ethanol extracts of the waxy wheat sprouts; NSW, water extracts of the normal wheat sprouts; NSE, 80% ethanol extracts of the normal wheat sprouts. Values are shown as mean ± SD (n ≥ 3). Values with a same letter (a–f) are not significantly different by Duncan's multiple range test ($p < 0.05$).

The flavonoid content of the extracts of WS and NS germinated for 7, 9, and 11 days is presented in Figure 1B. The flavonoid content of water and 80% ethanol extracts of both wheat extracts increased with germination time significantly as for the total phenolic content while the contents of 80% ethanol extracts of WS did not differ significantly between 7 and 9 days, and the water extracts of NS between 9 and 11 days. The flavonoid content of WS was found to be higher than that of NS. The flavonoid contents of 80% ethanol

extracts were higher than that of water extracts in each wheat sprout, in contrast to the total phenolic content. To determine whether water or ethanol is the effective solvent for extraction depends on the food type and target compound, and for the wheat sprouts in this study, water seems to be a highly effective solvent for phenolic compounds and 80% ethanol for flavonoids.

Phenolic compounds and flavonoids are natural antioxidants that are known to be responsible for the antioxidant activity of plant materials [21,22]. The major phenolic compounds of wheat grain are ferulic acid, catechin, epigallocatechin gallate and luteolin [23]. The total phenolic content of the extracts increased with germination time in both WS and NS, and this increase was also reported for Polish winter wheat cultivars germinated for 2–8 days [24], and for einkorn and emmer wheat grains until 12 days of growth [25]. Total phenolic content was also reported to be higher in wheat sprouts that germinated for one day than the wheat grains [2], implying an increase in phenolic compounds attributed to seed germination. Germination for 5 days have been shown to increase the phenolic content in the methanol extracts of wheat, barley, rye, and sorghum, but not in buckwheat and brown rice [23]. The presence of flavonoids in the water extract of commercial wheatgrass has previously been reported [26], and the flavonoid content was found to be increased with germination until 72 h in the 80% methanol extracts of wheat grains, which was also observed in barley, sorghum, and millet [27], showing consistency with the results of this study. As shown in the results, WS exhibited the same increase as that of NS in the flavonoid contents with germination time and higher flavonoid contents than NS, thereby implying higher antioxidant activity exhibited by WS than NS.

### *3.2. Antioxidant Activities*

The Antioxidant activity of the water and 80% ethanol extracts of Korean WS and NS germinated for 7, 9, and 11 days was measured in terms of DPPH and ABTS radical scavenging activities and reducing power. DPPH radical scavenging activity of water and 80% ethanol extracts of both the sprouts are presented in Figure 2A. The activities of water and 80% ethanol extracts increased with germination time in both wheat sprouts, and the activities of 80% ethanol extracts were significantly higher than those of water extracts in each wheat sprout. DPPH radical scavenging activity of both extracts was higher in WS than in NS until 9 days of germination, but were higher in NS at 11 days of germination. DPPH radical scavenging activity has been shown to increase with germination until 72 h in the 80% methanol extracts of wheat grains [27], and germination for 110 h increased this activity more than two times compared to wheat grains [28], and these results are concordant with the results of this study.

ABTS radical scavenging activity of the extracts of WS and NS is presented in Figure 2B. The activities of water and 80% ethanol extracts also increased with germination time, and the activities of 80% ethanol extracts were significantly higher than those of the water extracts, similar to that of DPPH radical scavenging activity. ABTS radical scavenging activity was lower in the water extracts of WS germinated for 9 days and higher in the 80% ethanol extracts of WS germinated for 7 days than those of NS; however, other extracts did not show such significant differences between WS and NS. In a previous study, ABTS radical scavenging activities of 80% methanol extract of wheat sprout was also found to be increased with germination until 120 h, although there was a reduction at 72 h [29]. The activity of the 70% ethanol extract of wheat germinated for 3 days was higher than that of the wheat grain [30].

The reducing powers of the extracts of WS and NS is presented in Figure 2C. The reducing power of the extracts increased with germination time, similar to the radical scavenging activities. The powers of 80% ethanol extracts were similar to those of water extracts in WS, but the activities of 80% ethanol extracts were higher than those of the water extracts of NS after 9 days of germination. Both extracts of WS exhibited higher reducing powers than those of NS at 7 days of germination, and there was no significant difference observed after 9 days. Kaur and Gill [27] reported that the reducing power

of 80% methanol extracts of wheat grains increased with germination until 72 h, as also observed in this study.

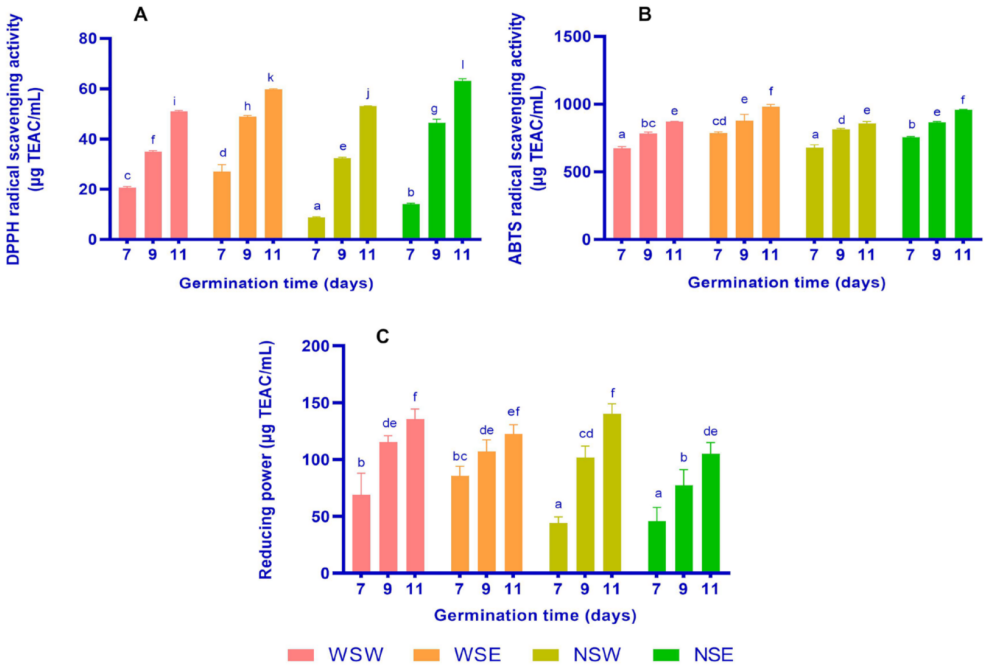


**Figure 2.** Antioxidant activities of Korean waxy and normal wheat sprouts germinated for 7, 9, and 11 days. (**A**) DPPH radical scavenging activity; (**B**) ABTS radical scavenging activity; (**C**) Reducing power. WSW, water extracts of the waxy wheat sprouts; WSE, 80% ethanol extracts of the waxy wheat sprouts; NSW, water extracts of the normal wheat sprouts; NSE, 80% ethanol extracts of the normal wheat sprouts. Values are shown as mean ± SD (n ≥ 3). Values with a same letter (a–l) are not significantly different by Duncan's multiple range test ($p < 0.05$).

The radical scavenging activity and reducing power are the main antioxidant mechanisms of phenolic compounds and flavonoids, which are known as the main plant antioxidants. The radical scavenging activity was determined through measuring the reduction of free or peroxide radicals produced during the oxidation process while the reducing power through the reduction of metal ions [31]. Although germination is known to be effective process to improve the antioxidant activity of grains, it is not applicable to all grains. Total phenolic content and radical scavenging activity were found to be similar before and after germination in oat and brown rice, and reduced after germination in buckwheat, which is contrary to the results in wheat [23]. Germination is an effective method to increase the antioxidant activities of both waxy and normal wheat grains, but WS usually exhibited higher activity than NS until 9 days of germination. This means that WS could be used as a better food material for antioxidant activity at the early stages of germination than NS.

### *3.3. α-Glucosidase Inhibitory Activity*

α-Glucosidase inhibitory activity of 80% ethanol extracts of Korean WS and NS germinated for 7, 9, and 11 days is presented in Table 1. α-Glucosidase is present in the brush border of the small intestine and hydrolyzes carbohydrates, such as maltose, into glucose [32]. Glucose is then absorbed, resulting in an increase in blood glucose, therefore, the inhibition on α-glucosidase exhibits a hypoglycemic effect, which is important for preventing and treating diabetes mellitus [33].

The water extracts of WS and NS did not show α-glucosidase inhibitory activity. The activity of 80% ethanol extract of WS germinated for 7 days was the lowest at 84.01% among all extracts, and there was no significant difference among the extracts except this one. All other extracts exhibited α-glucosidase inhibitory activity higher than 90%. In the previous studies, germination for 5 days was shown to increase α-glucosidase inhibitory activity of

wheat compared to non-germinated wheat grain [23], and feeding wheat sprout extracts reduced blood glucose levels in BALB/c mice as also mentioned previously [14], which is consistent with the results of this study. Germination did not improve the activities in oat, buckwheat, and brown rice [23], implying that germination is an effective process for improving the hypoglycemic effects of wheat, although not for all grains.

**Table 1.** α-Glucosidase inhibitory activities of 80% ethanol extracts of Korean waxy and normal wheat sprouts germinated for 7, 9, and 11 days.

| Germination Time | Glucosidase Inhibitory Activity (%) [1] | |
|---|---|---|
| | Waxy Wheat Sprout | Normal Wheat Sprout |
| 7 days | 84.61 ± 6.14 [a,2] | 90.85 ± 0.24 [b] |
| 9 days | 90.92 ± 0.38 [b] | 93.58 ± 0.85 [b] |
| 11 days | 94.19 ± 0.40 [b] | 94.47 ± 0.49 [b] |

[1] Values are shown as mean ± SD (n ≥ 3). [2] Values with a same letter ([a, b]) are not significantly different by Duncan's multiple range test ($p < 0.05$).

Phenolic compounds including quercetin, ferulic acid, and *p*-coumaric acid, are known to inhibit α-glucosidase activity [34]. Phenolic compounds also increase the absorption and uptake of glucose into peripheral tissues and insulin secretion lowering the level of blood glucose [35]. Non-starch polysaccharides with α-glucosidase inhibitory activity are also known to be present in wheat, and the isolated phosphatidic acids, including 1,2-dilinoleylglycerol-3-phosphate and 1-palmitoyl-2-linoleoyl glycerol-3-phosphate, in wheat germ exhibit high α-glucosidase inhibitory activity [34]. These compounds might be responsible for α-glucosidase inhibitory activity of wheat sprout examined in this study, although not for all.

### *3.4. Nitric Oxide Production Ratio*

NO production ratio of MEF cells treated with water and 80% ethanol extracts of Korean WS and NS germinated for 7, 9, and 11 days is presented in Figure 3. NO production ratio of MEF cells treated with WS and NS was measured through comparing NO production with and without cytokine stimulation in this study. NO production ratio increased with the application of WS for up to 9 days of germination, but decreased at 11 days. There was no significant difference in the ratio after the application of NS germinated for various time period. Treatment with the 80% ethanol extract increased NO production ratio more than the water extracts for WS. The ratio for WS was higher than that of NS, and even higher than that of the control after 9 days of germination.

NO is produced by the enzyme, inducible nitric oxide synthase (iNOS) during inflammation, which causes tissue damage, organ dysfunction and tumorigenesis, thereby leading to several acute and chronic diseases [36,37]. Therefore, NO production is used as a biomarker of the inflammatory response [38], and lower NO production indicates higher anti-inflammatory activity. The results indicate higher anti-inflammatory activity in NS than in WS, and an increase in inflammatory response by WS after 9 days of germination. Previously, 50% ethanol extract of commercial wheat sprout was shown to reduce NO production in raw 264.7 cells compared to the untreated cells [12], and the application of wheat sprout extracts to raw 264.7 macrophages inhibited the production of TNF-α, a biomarker of inflammation, for up to 5 days at 20 °C [39]. Whent et al. [40] reported a negative correlation between the level of IL-1β mRNA expression and contents of *p*-coumaric acid and α-tocopherol in wheat flour. Although there are no reports on the compounds responsible for the anti-inflammatory activity of wheat sprouts, *p*-coumaric acid and α-tocopherol could be one of the compounds exhibiting the activity in wheat sprout.

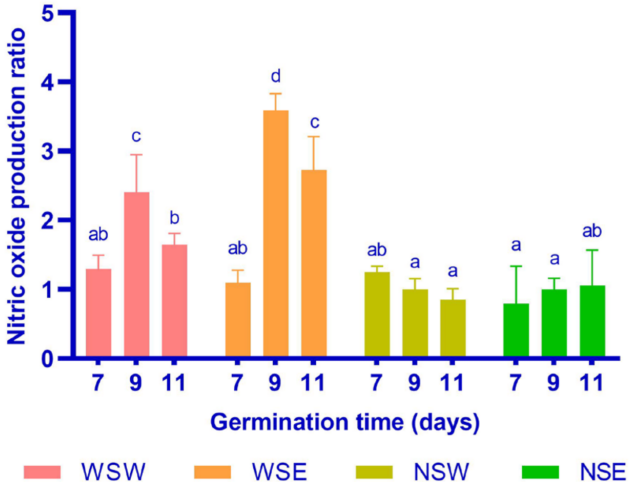


**Figure 3.** Nitric oxide production ratio in MEF cells treated with Korean waxy and normal wheat sprouts germinated for 7, 9, and 11 days. WSW, water extracts of the waxy wheat sprouts; WSE, 80% ethanol extracts of the waxy wheat sprouts; NSW, water extracts of the normal wheat sprouts; NSE, 80% ethanol extracts of the normal wheat sprouts. Values are shown as mean ± SD (n ≥ 2). Values with a same letter (a–d) are not significantly different by Duncan's multiple range test ($p < 0.05$).

### *3.5. Cell Viability*

The viability of Caco-2 cells treated with water and 80% ethanol extracts of Korean WS and NS germinated for 7, 9, and 11 days is presented in Figure 4. The cell viability after the treatment with the water extracts of WS and NS increased with germination time, and it was higher for the water extracts of WS than that of NS. Treatment with 80% ethanol extracts of WS decreased the cell viability with germination time, while that of NS did not exhibit a significant difference over time. The cell viability after the treatment with ethanol extract were higher for NS than WS after 9 days of germination, in contrast to the water extract. However, all extracts exhibited more than 90% cell viability, thereby implying that all extracts of WS and NS did not exhibit cell toxicity. The sample is considered non-cytotoxic if it does not reduce the cell viability below 85% [38].

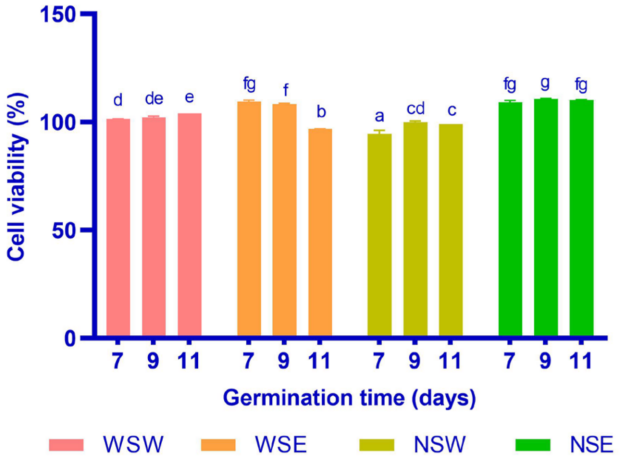


**Figure 4.** The viability of Caco-2 cells treated with Korean waxy and normal wheat sprouts germinated for 7, 9, and 11 days. WSW, water extracts of the waxy wheat sprouts; WSE, 80% ethanol extracts of the waxy wheat sprouts; NSW, water extracts of the normal wheat sprouts; NSE, 80% ethanol extracts of the normal wheat sprouts. Values are shown as mean ± SD (n ≥ 2). Values with a same letter (a–g) are not significantly different by Duncan's multiple range test ($p < 0.05$).

The human intestinal epithelium is an important barrier against the invasion of microorganisms during the intake of contaminated food [41]. Therefore, the enhancement of viability or non-cytotoxicity on these cells is beneficial for human health. Wheat aqueous extract was reported to increase the cell viability with an increase in the dose of the extract [42], but there have been no reports on wheat sprout. Apigenin, a flavonoid in plants, and arabinoxylan, a soluble dietary fiber, are known to promote cell viability [43,44] and are present in wheat and wheat sprout [23,43,45]. They may be partially responsible for the promoting effect of waxy and normal wheat sprouts on the cell viability.

### *3.6. Pearson's Correlation Coefficient*

Pearson's correlation coefficient between the total phenolic and flavonoid contents, and various bioactivities including antioxidant and anti-inflammatory activities and cell viability determined for WS and NS is exhibited in Figure 5. As mentioned previously, phenolic compounds and flavonoids are natural antioxidants of plant materials [21,22]. Therefore, positive correlations were found significantly between total phenolic and flavonoids contents with all antioxidant activities determined by three different assays, which are DPPH, ABTS, and reducing power assays. These three different assays also exhibited significant correlation between each other. The linear correlation between phenolics and flavonoid contents with radical scavenging activity and reducing power in plant material was reported previously [46].

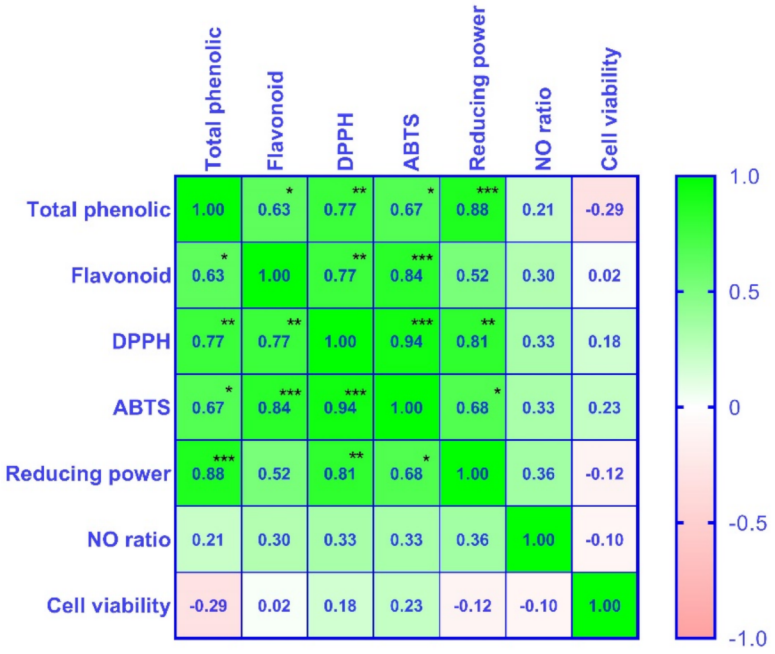


**Figure 5.** Pearson's correlation coefficient between the total phenolic and flavonoid contents, DPPH and ABTS radical scavenging activities, the reducing power, NO production ratio in MEF cells, and the viability of Caco-2 cells for Korean waxy and normal wheat sprouts (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The correlation coefficient of NO production ratio, a biomarker of inflammation response, with other factors were not significant and this demonstrated the absence of linear correlation with them. Although *p*-coumaric acid is reported to exhibit anti-inflammatory activity in wheat flour [40], it is assumed that other compounds which does not belong to phenolics, may affect anti-inflammatory activity in WS or NS. Cell viability also did not exhibit linear correlation with any other factors and this implies that the major compounds related to the effect promoting cell viability are not phenolic compounds.

## 4. Conclusions

Germination increased total phenolic and flavonoid contents, and antioxidant activity but did not α-glucosidase inhibitory and anti-inflammatory activity in WS and NS. WS exhibited higher flavonoid content, DPPH radical scavenging activity, reducing power, NO production ratio, and the viability of Caco-2 cells than NS. Total phenolic content and α-glucosidase inhibitory activity were not significantly different between WS and NS. As an extract solvent, 80% ethanol was generally found to be more efficient in extracting the functional compounds from wheat sprouts than water, except for the total phenol content and NO production ratio. These results imply that WS exhibits better bioactivities in antioxidant activity and less cell toxicity for water extract than NS and NS better in terms of anti-inflammatory activities. Therefore, the waxy wheat sprout could be used as a health promoting food like normal wheat sprout for a certain purpose. Although the results of this study support the benefit of using WS, further studies on the qualification and quantification of the compounds responsible for the bioactivities of WS and NS should be conducted to clarify the relevance between the compounds and their activities.

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