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Low-Temperature Effects on the Growth and Phytochemical Properties of Wheat Sprouts

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Abstract: Sprouting is associated with nutritional value, as microgreens stimulate the accumulation of health-promoting phytochemicals. The purpose of this study was to examine the growth rates and cell protection activity against oxidative stress in sprouts of seven wheat varieties, and to investigate the influence of low temperatures on their phytochemical characteristics. Among the seven wheat varieties (five Korean varieties, Australian standard white, and Chinese wild-type wheat germplasm), purple wheat (Ariheuk) had the fastest growth pattern for 8 days and provided the most protection to skin cells and hepatocytes against oxidative stress. Following low-temperature treatment (<4 °C) for 1–4 days, cold exposure had a similar effect on the growth of purple wheat sprouts during an 8-day period. However, growth was negatively affected by exposure to low temperatures for more than 5 days. Purple wheat sprouts treated with low temperatures for 4 days had considerably higher total polyphenol and total flavonoid contents, as well as a higher antioxidant capacity than untreated wheat sprouts. These findings suggest that low-temperature treatment promotes the expression of phytochemicals in purple wheat sprouts. Thus, purple wheat sprouts are considered a high-value crop that could be used as a functional food material.

Keywords: colored wheat; grass; temperature; sprout; polyphenol; antioxidant

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1. Introduction

Wheat is a key crop for meeting global food demand and is among the most widely consumed crops worldwide [1]. In Korea, wheat is the second-most consumed crop per capita after rice, and the market for baked goods is expanding as a result of westernization of the Korean diet [2]. The estimated wheat production for 2021–2022 in South Korea has been revised down by 11%, whereas that for imported wheat has been raised by 12% [3]. Korea is largely reliant on wheat imports from the US, Australia, and Canada; domestic production accounted for <1% of all wheat production in 2020 [4]. The consumption of functional foods containing dietary fiber and antioxidants has increased due to growing consumer interest in health [5]. As part of a campaign by the National Institute of Crop Science to develop value-added grains, the Rural Development Administration produced the purple wheat variety, 'Ariheuk', to enhance domestic wheat production and consumption in Korea [2]. 'Ariheuk' wheat is rich in minerals, and its antioxidant capacity is superior to that of common wheat [2]. Several studies have demonstrated that purple wheat has greater antioxidant properties than red and white varieties [6,7].

Microgreens and sprouts have stimulated research in various fields [8]. Sprouting is associated with higher seed nutritional value, as it induces the accumulation of health-promoting phytochemicals [9]. Recent studies have demonstrated that wheat sprouts may be valuable for developing functional foods due to their high free phenolic acid contents [10,11]. Wheat sprouts contain a potent antioxidant cocktail of enzymes, reducing glycosides, and polyphenols that have remarkable radical scavenging properties [12]. In addition, abiotic stress cultivation conditions, such as low temperatures, also induce the accumulation of phenols and flavonoids [9]. Reactive oxygen species (ROS) form particularly readily at low temperatures [13]; however, plants strictly regulate ROS generation by recruiting antioxidants to protect cells from oxidative stress [1,14].

Wheat sprouts processed into powder or juice are widely consumed in the US and Europe as nutritional supplements and for metabolic disease prevention [15]. Sprouts have also become popular as functional foods in Korea in recent years. Extensive studies have demonstrated the nutritional value and antioxidant potential of sprouts of legumes such as soybean; however, few studies have examined sprouts of Korean wheat varieties. Therefore, in this study, we determined the growth patterns of sprouts of seven wheat varieties (five Korean varieties, Australian standard white, and Chinese wild-type wheat), and their potential to protect cells from oxidative stress. Furthermore, the effect of low-temperature treatment on the growth traits and phytochemical content concerning the antioxidant capacity of wheat sprouts was explored.

2. Materials and Methods

2.1. Materials

The following materials were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA): Folin–Ciocalteu (FC) reagent, phosphate-buffered saline (PBS), sodium carbonate (Na_2CO_3), gallic acid, aluminum trichloride ($AlCl_3$), sodium hydroxide (NaOH), Trolox, catechin, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium persulfate ($K_2S_2O_8$), dimethyl sulfoxide (DMSO), and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS). HyClone (Logan, UT, USA) provided Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin (pen-strep). Fetal bovine serum (FBS) was supplied from Grand Island Biochemical Co. (Grand Island, NY, USA). DoGenBio (Seoul, Korea) provided EZ-Cytox. Ethanol (EtOH), methanol (MeOH), and purified water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Wheat Sprouts

The Korean wheat cultivars 'BaekJoong' (IT227093), 'Baekkang' (IT332201), 'Saekeumkang' (IT332202), 'Jeonju391', and 'Ariheuk' (KCTC18591P) were supplied by the National Institute of Crop Science (Rural Development Administration, Korea). Two non-Korean cultivars, Australian standard white (ASW) and Chinese wild-type wheat germplasm (Heuk1, K253304), were compared with the Korean cultivars. Wheat samples were washed and soaked in distilled water for 16 h. The soaked wheat was placed in dishes (25 cm $\times$ 30 cm) covered with wet absorbent cotton for 2 days for germination (reaching ~1 cm in length). Sprouting then proceeded for 8 days under controlled conditions in an incubator with a relative humidity of 60–70% and a 16 h/8 h photoperiod at 22 °C. Cultivation was conducted in triplicate. At least 20 sprouts per plot were randomly selected for sprout length measurements (bottom to tip). Germinated wheat (1 cm) was exposed to <4 °C for 1–6 days, and then cultured in an incubator at 22 °C. Then, the sprouts were harvested, freeze-dried, and powdered. The powdered samples were extracted for 16 h in MeOH and the supernatant was concentrated, freeze-dried, and stored at −20 °C for further study.

2.3. Cell Viability

Freeze-dried wheat extract samples were dissolved in DMSO and diluted with growth medium to the desired concentration. Each sample was prepared in triplicate. The final concentration of DMSO was reduced to 0.1%. Control wells were treated with the same volume of medium containing 0.1% DMSO. Hs68 and HepG2 cell lines (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM supplemented with 1% pen-strep solution and 10% FBS. Then, serum-free DMEM was used to incubate Hs68 cells at approximately 90% confluence. The cells were covered with PBS and exposed to ultraviolet

(UV) B light (30 mJ/cm^2). Following UVB exposure, the cells were cultured with or without extracts from each wheat sprout variety for 24 h. The control group was prepared in the same manner, but without UVB irradiation or sample treatment. HepG2 cells were cultured in the exponential growth phase for 24 h and treated with 3% EtOH after incubation with each of the wheat sprout extracts [16,17]. The culture medium was replaced with fresh medium containing 0.1 µL/mL of EZ-Cytox solution to determine cell viability. The cells were incubated at 37 °C for 2 h. Then, absorbance was confirmed at 450 nm. The viability of cells (%) was determined using the following equation: (Absorbance of treatment group/Mean absorbance of untreated control) × 100.

2.4. Quantification of Phenolics and Flavonoids in Wheat Sprouts

Total phenolic (TP) content was analyzed using the FC colorimetric method [18], with some modifications. Briefly, 0.2 mL of extracts was mixed with 1.0 mL of 2% aqueous Na_2CO_3 solution, and then with 0.1 mL of 1 N FC reagent. After 30 min of incubation, the absorbance of the resulting blue color was measured at 720 nm. The standard was gallic acid, and TP content is indicated as mg gallic acid equivalents (GAE)/g of sample (dry basis). Total flavonoid (TF) content was measured using a modified colorimetric method [19]. A 0.2 mL aliquot of extracts was added to a tube containing 0.075 mL of 5% $NaNO_2$ solution. Then, 0.15 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added to the mixture. After 5 min, 0.5 mL of 1 M NaOH solution was added. The absorbance was determined immediately at 510 nm. TF content is indicated as mg of (+)-catechin equivalents (CE)/g of sample (dry basis).

2.5. Radical Scavenging Activity

The DPPH and ABTS$^{\bullet+}$ assays were performed with modifications [20] to assess radical scavenging activity. Briefly, 1.0 mL of 0.2 mM DPPH solution was added to 0.2 mL of the extract. Thirty minutes later, absorbance was measured at 517 nm. ABTS (7.4 mM) was reacted with 2.6 mM $K_2S_2O_8$ solution and allowed to stand in the dark at room temperature overnight to generate ABTS radicals. The ABTS radical solution was diluted to yield an absorbance of 1.0 at 735 nm. Then, 0.2 mL of the extract was mixed with 1.0 mL of ABTS solution, and absorbance was measured at 735 nm after 60 min. The DPPH and ABTS radical scavenging effects of the extracts are expressed as Trolox equivalent (TE)/g of sample (dry basis).

2.6. Statistical Analysis

All sprout cultivation experiments and total phenolic, flavonoid, and radical scavenging activity measurements were conducted in triplicate. Differences among means were evaluated using independent Student's *t*-tests and a one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests with SPSS v20.0 software (IBM Corp., Armonk, NY, USA). Statistical significance was evaluated at a level of p-value < 0.05.

3. Results and Discussion

3.1. Wheat Sprout Characteristics Differed among Varieties

3.1.1. Wheat Sprout Growth Rates

Sprouts growth patterns were evaluated in the five Korean varieties to identify potential cultivars for use as wheat sprouts. During the past decade, 'BaekJoong' has mainly been used for noodles, whereas 'Baekkang' and 'Saekeumkang' are new high-yield, high-quality varieties used for bread and noodle production, respectively. 'Jeonju391' is considered a regional yield trial elite line wheat. 'Ariheuk' is a purple wheat hybrid generated by crossing Chinese wild-type wheat and Korean Shinmichal wheat. 'Heuk1' is a wild-type wheat germplasm from China. ASW has accounted for >47% of the imported wheat consumed in South Korea since 2016 [21]. Overall, the growth rates of Korean wheat sprouts were higher than those of non-Korean wheat varieties, Heuk1 (No. 1) and ASW (No. 2) (Figure 1). Interestingly, among the seven varieties, purple wheat (No. 7) showed remarkable growth from days 4 to 8. On day 8, purple wheat sprouts had an average length of 17.8 cm, whereas the other varieties ranged from 11.5 to 13.8 cm. Sprout germination and growth rates varied depending on the seed coat color [22,23]. Colored wheat variety sprouted more rapidly and had higher yields than a number of Korean domestic wheat varieties evaluated in our previous study [24]. Because colored seeds absorb water quickly, they have higher germination rates than non-colored seeds [25,26]. Anthocyanins in colored wheat have been demonstrated to affect sprouting rates [23]. Cyanidin-3-glucoside and peonidin-3-glucoside, which have not been identified in common wheat, were present in purple wheat ('Ariheuk') [27]. However, detailed investigations of the genetic factors affecting yield and growth patterns are required.

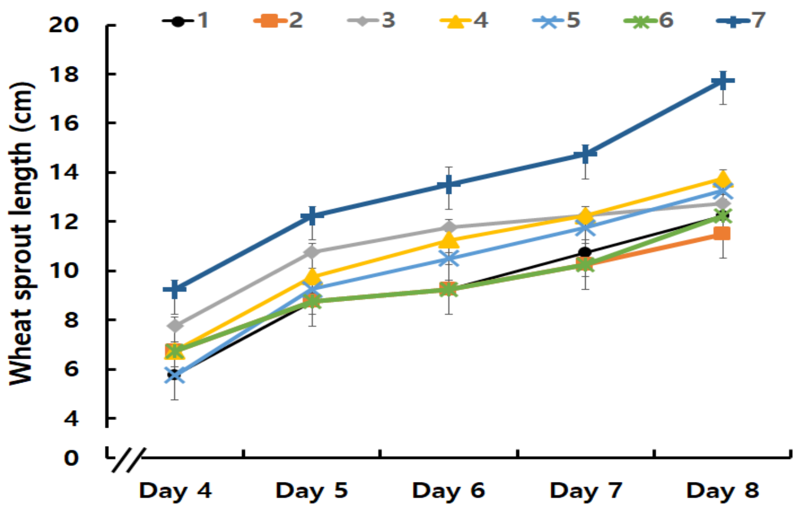

Figure 1. Effects of wheat variety on sprout length. (1) 'Heuk1'. (2) 'Australian standard white'. (3) 'BaekJoong'. (4) 'Baekkang'. (5) 'Saekeumkang'. (6) 'Jeonju391'. (7) 'Ariheuk' (purple wheat).

3.1.2. Wheat Sprouts Protect Cells against Oxidative Stress

Wheat sprouts are a major source of antioxidant molecules, which protect against age-related alterations [28]. Oxidative damage, which is linked to multiple alterations in cell structure and function, triggers chronic disease [29]. In this study, we detected a protective effect of wheat sprouts of the seven cultivars on human dermal fibroblasts (Hs68) and hepatocytes (HepG2) exposed to oxidative stress (Figure 2). Exposure to sunlight causes oxidative stress, and UVB causes photodamage to skin by stimulating ROS generation [30]. The viability of UVB-exposed Hs68 cells decreased significantly compared to the untreated control group (Figure 2A). However, when UVB-exposed cells were treated with wheat sprouts, cell viability increased significantly (ASW, 'Baekkang', 'Saekeumkang', and 'Ariheuk'). To determine the hepatocyte protective effect of wheat sprouts derived from the seven cultivars, we investigated whether each wheat sprout cultivar recovered the cell viability of EtOH-treated HepG2 cells. HepG2 cells exposed to EtOH suffer oxidative damage [29,31]. EtOH-treated cells had significantly reduced viability compared to the untreated control group, whereas those stimulated with EtOH and given wheat sprouts had significantly higher viability than the EtOH control group (Figure 2B). In hepatocytes, purple wheat sprouts (No. 7) had a higher cell protective effect against oxidative stress, providing comparable protection. 'Baekjoong' sprouts have been reported to reduce oxidative stress [15]; however, no such studies have examined the wheat sprouts of multiple cultivars. Among the varieties evaluated in this study, 'Ariheuk' is a colored wheat cultivar, whereas the others, including 'Baekjoong', produce white wheat. The total anthocyanin, quercetin, and pelargonidin contents are significantly higher in purple wheat than in blue or yellow wheat sprouts, suggesting that purple wheat sprouts are more promising than those of other colored wheat genotypes as a source of functional food [10]. Our comparison of multiple wheat varieties confirmed the potential of employing purple wheat ('Ariheuk') sprouts as a functional food material.

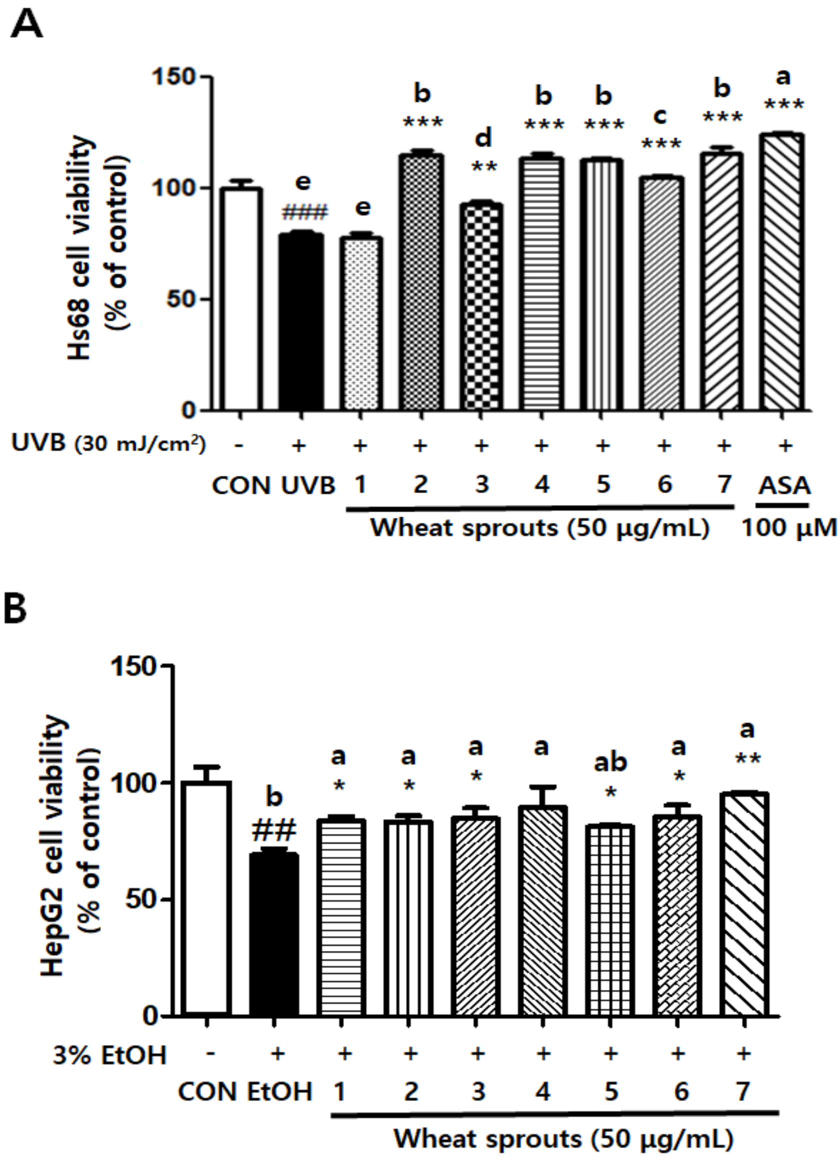

Figure 2. Effects of wheat sprouts of multiple cultivars on oxidative stress-stimulated cells. (**A**) Hs68 dermal fibroblasts exposed to ultraviolet B (UVB) irradiation (30 mJ/cm^2). (**B**) Hepatoma HepG2 cells were stimulated with 3% EtOH. The cell protective effects of the wheat sprouts were assessed in terms of percent cell viability. 1, 'Heuk1'; 2, 'Australian standard white'; 3, 'BaekJoong'; 4, 'Baekkang'; 5, 'Saekeumkang'; 6, 'Jeonju391'; 7, 'Ariheuk' (purple wheat). CON, untreated control; ASA, ascorbic acid. UVB- and EtOH-stimulated groups without sample treatment were compared with each of the indicated groups using independent Student's *t*-test ($^{##}$ $p < 0.01$; $^{###}$ $p < 0.001$ vs. untreated control group; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. stimulated group with sample treatment). Different letters represent significant differences between stimulated groups ($p < 0.05$; ANOVA, followed by Duncan's multiple range test).

3.2. Effects of Low-Temperature Treatment on Purple Wheat Sprout Properties

3.2.1. The Growth Rate of Purple Wheat Sprouts Depends on Duration of Low-Temperature Exposure

Sprouting of edible seeds is an effective strategy to promote the content of health-promoting compounds such as vitamins, which are important in the human diet [32]. Temperature can influence the accumulation of phytochemicals in sprouts [33]. Exposing seeds to cold stress during germination typically delays seed germination rates and suppress physiological processes [23,34]. Securing sprout production is necessary for ensuring their health benefits. Therefore, in this experiment, we evaluated the growth patterns of purple wheat sprouts to determine an adequate low-temperature treatment period for wheat sprouts. We observed the effects of 1–6 days of low-temperature treatment (T1–T6) on sprout growth patterns (Figure 3). Purple wheat sprout lengths varied depending on the duration of low-temperature treatment relative to the control (Figure 3). Unlike 4-day low-temperature treatment (T1–T4), treatment for more than 4 days (T5 and T6) harmed growth (Figure 3A,B).

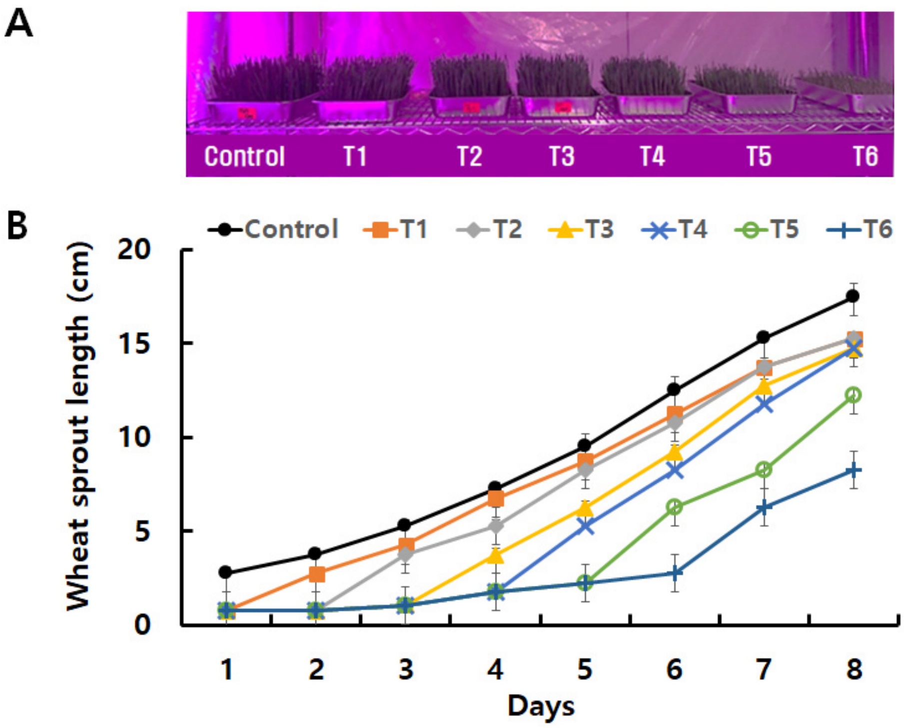

Figure 3. (**A**) Growth of purple wheat sprouts on day 8, and (**B**) sprout growth trends throughout the low-temperature treatment period (T1–T6). Control, untreated control; T1–T6, number of days at low temperature (<4 °C).

3.2.2. Phytochemical Properties of Low-Temperature-Treated Purple Wheat Sprouts

A previous study reported that cold cultivation conditions increased the expression levels of flavonoid biosynthetic genes in purple wheat [23]. In this study, we observed variation in purple wheat sprout TP and TF contents as the duration of low-temperature treatment was extended to 4 days (Figure 4A). Low-temperature treatment enhanced phytochemical properties including the quantity and activity of the antioxidant compounds. Increases in TP and TF contents appear to have elevated the antioxidant capacity of the sprouts (Figure 4B). These findings demonstrate that the nutritional quality of colored wheat sprouts was improved by modulating the temperature during sprouting. Based on ROS scavenging enzyme activity and the regulation of cold-responsive genes, darker seed coats were particularly responsive to cold acclimation [23]. Cold stress affects both non-enzymatic (e.g., flavonoids) and enzymatic (e.g., superoxide dismutase and catalase) antioxidants [14]. Unfortunately, this study was limited to identifying non-enzymatic antioxidants and activities; therefore, further study is required to verify changes in enzymatic antioxidants and related genes.

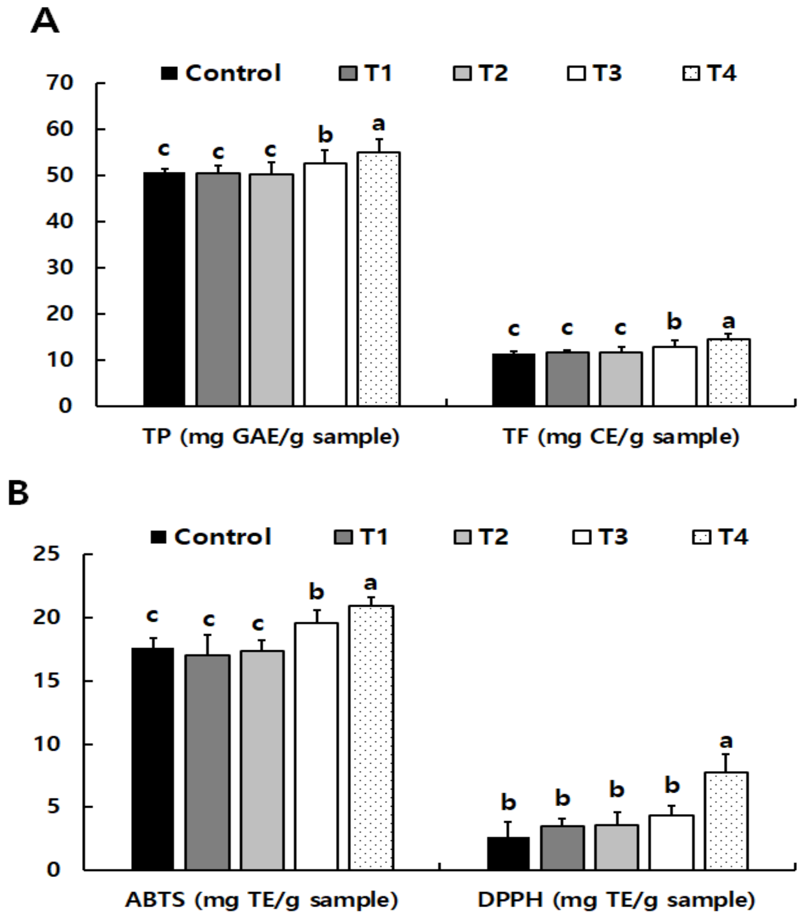

Figure 4. (**A**) Total phenolic and flavonoid contents, and (**B**) ABTS and DPPH radical scavenging activity of purple wheat sprouts, were affected by the duration (T1–T4) of low-temperature treatment (<4 °C). TP, total phenolic content; GAE, gallic acid equivalent; TF, total flavonoid content; CE, catechin equivalent; TE, Trolox equivalent. According to ANOVA, different letters indicate significant differences between groups ($p < 0.05$; ANOVA, followed by Duncan's multiple range test).

4. Conclusions

The results of this study demonstrate that 'Ariheuk', a purple wheat cultivar from Korea, had a higher sprout growth rate than common wheat sprouts, and a higher antioxidant capacity in cells under oxidative stress. Low-temperature treatment (<4 °C) for up to 4 days was effective for maintaining wheat sprout growth while enhancing the antioxidant content and capacity. These findings will aid the development of wheat varieties that provide high-value agricultural products. In particular, purple wheat sprouts processed at low temperatures could be employed as functional food ingredients.

5. Patent

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