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ARTICLE INFORMATION	Fill in information in each box below
Article Title	Biochemical and antioxidant activity of yogurt supplemented with paprika juice of different colors
Running Title (within 10 words)	Characteristics of yogurt supplemented with paprika juice of different colors
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	
Author contributions (This field may be published.)	Conceptualization: Hong H, Son YJ, Kwon SH, Kim SK. Data curation: Hong H, Son YJ, Kwon SH, Kim SK. Formal analysis: Son YJ, Kwon SH. Methodology: Hong H, Son YJ, Kwon SH. Software: Son YJ, Kwon SH. Validation: Hong H, Kim SK. Investigation: Hong H, Son YJ, Kwon SH, Kim SK. Writing - original draft: Hong H, Son YJ, Kim SK. Writing - review & editing: Hong H, Kim SK.
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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Abstract

Paprika is known to contain polyphenolic compounds that have good antioxidant properties. This study was conducted to investigate the benefits of adding paprika juice of different colors to yogurt and to determine how paprika affects the quality characteristics of yogurt. Stirred yogurt samples supplemented with different levels of red, orange, or yellow paprika juice were inoculated with mixes of *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *Bulgaricus.* Paprika addition decreased the pH but increased titratable acidity, lactic acid bacteria counts, total polyphenol content, levels of vitamin A and C, and antioxidant activity. Proteolysis and viscosity of paprika-containing yogurts were significantly higher than those of the control yogurt without paprika juice ($p < 0.05$). In particular, the viscosity of red paprika yogurt was significantly higher than that of yogurts containing 5% orange and yellow paprika juices (each $p < 0.05$). The antioxidant activity of the methanol extract of the yogurt containing 2.5% orange paprika juice was the highest. Storage at 4°C for 15 days only slightly altered lactic acid bacteria counts, antioxidant activity, and total polyphenol content of paprika yogurt. These results indicate that paprika could be used as a natural food additive for the development of functional yogurts.

Key words: yogurt, paprika, polyphenol, vitamin A, vitamin C

Introduction

Fermentation is a chemical process in which enzymes break down organic substances into smaller compounds (Schlesinger and Bernhardt, 2013). A result of fermentation is more digestible, stable, and flavored foods, with enhanced nutritional values (Bernaert et al., 2013; El-Abbadi et al., 2014). Yogurt is a fermented milk product obtained from the fermentation of *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* (Wasilewska et al., 2019). It was first produced in the Middle East but has spread worldwide (Wang et al., 2013); it contains higher levels of protein, vitamin B, and minerals including calcium, magnesium, potassium, and zinc than milk (El-Abbadi et al., 2014). Furthermore, yogurt is a suitable dairy product for people who are lactose intolerant, as the lactose in milk is converted to lactic acid during fermentation (Savaiano, 2014).

Paprika is the ground spice made from the botanical fruit, pepper, consumed as a vegetable; it belongs to the *Solanaceae* family, genus *Capsicum*, and the species *C. annuum* L, and is one of the most consumed vegetables globally (Di Cagno et al., 2013). It has six subspecies and is referred to as sweet pepper, bell pepper, and paprika, depending on the country (Di Cagno et al., 2013). *Capsicum annuum* species contain a high concentration of carotenoid pigments, responsible for the yellow, orange, and red colors of the vegetables (Halah and Nayra, 2011). Paprika pigments are composed of capsanthin and capsorubin, which are xanthophyll carotenoids (Arimboor et al., 2015). Paprika is known as a health food because it is rich in vitamin A, C, E, and flavonoids (Kantar et al., 2016; Hassan et al., 2019). The phytochemical content and physiological activities of paprika differ depending on the color of the paprika (Kim et al., 2016). Kim et al. (2016) reported that 100 g of dried red and green paprika contained 70 and 10 mg of carotenoids, 50 and 100 mg of tocopherol, and 750 and 700 mg of polyphenols, respectively. Moreover, green paprika contains high levels of chlorophyll, yellow and orange paprika contain lutein, zepaxanthin, and β-carotene, while red paprika contains carotenoid pigments, such as capsanthin and capsorubin (Marin et al., 2004; Arimboor et al., 2015). Paprika has received attention as a functional food and as a food additive, with various studies reporting that the phytochemicals in paprika exert antioxidant (Halah and Nayra, 2011), anti-cancer (Šaponjac et al., 2014), anti-inflammatory (Chen and Kang, 2013), anti-obesity (Maeda et al., 2013), and anti-arteriosclerotic activities (Tsui et al., 2018). However, to date, there has been no active study on the colors of paprika, especially with yogurt and green paprika juice combined, and no research has been reported on the manufacturing of paprika color-specific yogurts.

Therefore, this study investigated the biochemical and active antioxidants characteristics of yogurt with red, yellow, and orange paprika juice; while observing the consequent quality changes under storage, this study presents basic information on the role of these food additives in enhancing food functionality.

Materials and Methods

Preparation of paprika juice and stirred yogurt

Red, orange, and yellow paprika were purchased from a local market in Seoul, Korea at winter season. For preparing the paprika juice, the paprika were washed with tap water several times and were dehydrated. The paprika juice for each color was prepared by grinding in a juicer (DA 5000, Dasung Artron, Paju, Korea), and the solid particles were separated by filtering, using 4-layer sterile gauzes (JASCOR, Seoul, Korea) in the clean bench. Yogurt was prepared by adding skim milk powder (4%), pectin (0.2%), and white sugar (1%) to the milk and homogenizing using a Homogenizer T 25 (Janke and Kunkel type, Ika, Germany) for 15 min. The yogurt was inoculated with mixed strains of *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp.

Bulgaricus (YO-MIXTM 496 LYO 250 DCU, DANISCO, Copenhagen, Denmark) with 2.5 or 5% of each color paprika juice added. The yogurt was fermented at 43°C for 5 h and stored at 4°C.

pH and fermentation rate

The pH of the homogenized yogurt was measured using a digital pH meter (ISTEC 735P, Korea). The fermentation rate was determined using equation 1:

$$Fermentation\ \ rate\ \ (\%) = \frac{(A-B)}{fermentation\ \ time} \times 100 \qquad (1)$$

where, A was the pH before fermentation and B was the pH after fermentation.

Titratable acidity (TA)

The TA was measured by diluting the paprika yogurt sample to 1:9 with tertiary distilled water, as described previously (Kang et al., 2018). The sample was titrated with 0.1 N NaOH, with continuous stirring, to an end point of pH 8.3, recording the volume of NaOH (mL) used. The amount of acid produced during fermentation was calculated using equation 2:

$$TA\ \ (\%\ \ Lactic\ \ acid) = Dilution\ \ factor \times VNaOH \times 0.1 \times 0.009 \times 100 \qquad (2)$$

where, VNaOH was the volume of NaOH required to neutralize the acid.

Measurements of the lactic acid bacteria (LAB) in the paprika yogurt

LAB were counted using *Streptococcus thermophiles* (ST) agar plates composed of 10 g tryptone, 1 g sucrose, 5 g yeast extract, and 2 g K_2HP0_4 (Saccaro et al., 2011), after incubation for 48 h at 37℃, and expressed as CFU/g. Samples of the paprika yogurt were diluted; the diluted solutions from each step were spread on the ST agar plates.

Measurement of viscosity

The viscosity of the paprika yogurt was measured using a Bookfield-Viscometer (Model LVDV-

E, Brookfield Engineering Lab. Inc., Middleboro, MA, USA) with spindle No. 63 at 5 rpm. The measurements were recorded three times, at intervals of 30 s after 3 min.

Syneresis and peptide concentrations

Syneresis measured the weight of the supernatant following centrifugation (640×g, 4°C, 20 min) of samples, and was calculated using equation 3:

$$Syneresis\ (\%) \ = \ \left(\frac{weight\ \ of\ \ the\ \ supernatant\ \ (g)}{sample\ \ weight\ \ (g)}\right) \times \ 100 \qquad (3)$$

The amino acid content was determined as described previously (Goodno et al., 1981). Samples (30 μL) were mixed with 1 mL of OPA reaction solution and reacted for 2 min at room temperature. The absorbance was measured at 340 nm with tryptone used as the reference.

Color measurement

Color analysis of the yogurt was performed using a Chroma meter (MINOLTA CHPOMA METER CR-210). The measurements were conducted under artificial light to minimize the effects of daylight. The color parameters L* (lightness), a* (red / greenness), and b* (yellow / blueness) of the yogurt samples were evaluated according to the International Commission on Illumination (CIE) L*a*b* system.

Determination of total polyphenol and flavonoid contents

After dissolving the paprika yogurt in water and 95% methanol at a ratio of 1:9, samples were extracted at room temperature for 10 h. Folin-Ciocalteu reagent (0.2 mL, 1N) was added to each sample (0.2 mL) and thoroughly mixed. The solution was incubated at room temperature for 3 min, and 1 N Na_2CO_3 (400 μL) was added, mixed thoroughly, and further incubated at room temperature for 90 min in the dark. Absorbance of each sample was measured at 725 nm (UV-1601, Shimadzu, Kyoto, Japan) after mixing with 2 mL of distilled water (Wei, 2011). Known concentrations of gallic acid (Sigma-Aldrich, Schnelldorf, Germany; 5-60 μg/mL in ethanol) were treated as the yogurt extracts. Regression lines of the gallic acid standard were used to determine the total phenolic content of yogurt water extract using μg gallic acid equivalents (μg GAE)/mL.

The total flavonoid content was measured according to a previous method by Abeysinghe et al. (2007). The sample (0.42 mL) was mixed with 2.1 mL diethylene glycol and 0.21 mL 1 N NaOH. The mixture was incubated for 1 h in a water bath at 37°C; the absorbance was measured at 420 nm using quercetin (Sigma-Aldrich, St. Louis, MO, USA) as the standard.

Determination of vitamin A and C levels

The vitamin A and C levels were measured using high performance liquid chromatography (HPLC) at the Korea Testing and Research Institute, with retinol and ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) used as the standards. Vitamin A levels were analyzed using HPLC (Waters 616 system, Waters Co., Milford, MA, USA) equipped with SP column C18 (150 × 4.6 mm). Under the analysis conditions, column temperature was 35°C, flow rate 0.6 mL/min, run time 45 min, and injection volume 15 µL. The mobile phase comprised water:ethanol (20:80, v/v) and vitamin A was detected at 340 and 460 nm. Vitamin C levels were analyzed using HPLC (Waters 2695 system, Waters Co., Milford, MA, USA) equipped with SP column C18 (250 x 3.0 mm). Under the analysis conditions, column temperature was 40°C, flow rate 0.7 mL/min, run time 45 min, injection volume 20 µL, and detection wavelength 254 nm. Mobile phase A comprised water:methanol (97.5:2.5, v/v) and H solution 20 mL (heptanesulfonic acid (1 g) + water (10 mL) + acetic acid (10 mL)). Mobile phase B comprised water:methanol (50:50, v/v) and H solution (20 mL).

Antioxidant activity

Free radical scavenging activities were measured using 2-diphenyl-2-picrylhydrazyl (DPPH) photometric assay, as described previously (Apostolidis et al., 2007), with some modifications. One milliliter of a sample was mixed with 0.25 mL of 0.15 mM DPPH (Sigma-Aldrich, St. Louis, MO, USA). The mixture was kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a spectrometer (UV-1601, Shimadzu, Kyoto, Japan). Inhibition of DPPH oxidation (%) was calculated using equation 4 (Apostolidis et al., 2007):

$$Scavaging\ activity\ (\%) = \left(\frac{A_{control} - A_{extract}}{A_{control}}\right) \times 100 \qquad (4)$$

where, A was the absorbance at 517 nm.

Changes in paprika yogurt during storage

The samples were stored in a 4°C refrigerator for 15 days and changes in pH, TA, LAB count, viscosity, yield, amino acid concentration, antioxidant activity, and color were assessed at 5-day intervals.

Statistical analysis

Data are expressed as means ± standard deviation (SD) of at least three replicates. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA; SPSS 20) followed by Duncan's multiple range test and Student t-test for mean comparison. Statistical significance was considered at $p < 0.05$.

Results and Discussion

Characteristics of paprika yogurt according to the paprika juice levels

As shown in Table 1, the pH of the paprika yogurt decreased to 4.44–4.53 by increasing the content of paprika juice. The pH of the paprika yogurt with 5% yellow paprika juice was significantly lower than that of the yogurt without paprika ($p < 0.05$). The pH of the paprika yogurt with 5% paprika orange paprika was significantly higher than that of the other yogurts ($p < 0.05$). The pH of the yogurt with 2.5% red paprika juice was significantly higher than that of the other yogurts with 2.5% paprika juice ($p < 0.05$). The pH of the paprika juice used in this study was 4.9–5.2 (data not shown), indicating weak acidity. This indicated that the addition of paprika juice resulted in an increase in the acidity of the paprika-containing yogurt. The TA value was 0.72% in the yogurt without the paprika juice but was significantly higher for the red, orange, and yellow paprika yogurts (each $p < 0.05$) with TA values of 0.88–0.95%. These results were similar to previous reports of the pH and TA of stirred yogurt with added fermented pepper powder as 4.3–4.61 and 0.8–0.95%. respectively (Yu et al., 2014; Kang et al., 2018). According to Kim et al. (2011), the total organic acid content was 12,941.9 and 15,746.56 mg/100 g dry weight in red and green paprika, respectively. Thus, our results might be attributed to an increase in the organic acid content because of the addition of the paprika juice.

The LAB count in yogurts without paprika juice was 10.3–10.4 $\log_{10}$ (CFU/g). As the content of red and orange paprika juice increased, LAB increased to 10.45–10.85 $\log_{10}$ (CFU/g), a count significantly higher than that of control yogurt ($p < 0.05$). The LAB counts were greater than 9.09–9.35 $\log_{10}$ (CFU/g) when red and green peppers were added to yogurt (Kang et al., 2018) and were over 100 times higher than the standard value prescribed by the Korea Food Hygiene Regulations (2001). In this study, there was little change in LAB between the red, orange, and yellow paprika yogurts. These results demonstrate that all three paprika juices had positive effects on the growth of probiotic bacteria. According to the USDA (2019), raw red paprika has 6 g of carbohydrates and 4 g sugar per 100 g, while Jovanovic-Malinovska et al. (2014) reported that the carbohydrates in paprika are mostly sugars, such as glucose and fructose, which are sources of nutrients for probiotic bacterial growth.

Effect of paprika juice on peptide concentration, syneresis, and viscosity

The protein digestion of paprika yogurt without paprika juice was 0.11 mg/mL (Fig. 1A). However, as the amount of paprika juice increased, the peptide concentrations significantly increased from 0.11 to 0.21 mg/mL. Lorusso et al. (2018) reported that LAB increase the concentration of free amino acids. Syneresis in paprika yogurt without paprika juice averaged 28.3%, a value significantly lower than that found in the yogurt with 5% orange (34.98%) and yellow paprika juice (35.35%) (Fig. 1B). The gel network structure in yogurt is a relatively dynamic system, composed of casein, denatured whey proteins, and calcium phosphate crosslinks and is affected by many chemical and technological factors (i.e. dry matter content, enzyme activity, heat treatment, incubation temperature, and pH) (Lee and Lucey, 2010). Furthermore, Ziarno and Zareba reported that lower pH values result in higher syneresis (2019). The viscosity of the paprika yogurt with 2.5 and 5% paprika juice was 954 and 2,296 cp, respectively, values that were significantly higher than the viscosity of the yogurt without paprika juice ($p < 0.05$) (Fig. 1C). These results were inconsistent with reports of Kang et al. (2018) that the viscosity of yogurt decreased upon addition of fermented red or green pepper juice. Our results indicated that by increasing the levels of paprika juice, the pH value of the yogurt dropped. Similarly, Arioui et al. (2017) who observed that the viscosity of yogurt increased by lowering the pH. Sinaga et al. (2017) reported that changes in pH affect the casein micelle size and the gelation properties, as micelles swell in alkaline pH. These conditions weaken the cohesive interactions between the micelles, disrupting the hydrophobic bonds among the caseins, and eventually dissociating the casein micelles (Madadlou et al., 2009). Therefore, we believe that the addition of paprika juice increases LAB count, free amino acid level, and syneresis, but decreases the pH, leading to an increase in viscosity.

Effect of paprika juice on color change

Table 2 shows the changes in the color of yogurt containing 2.5 and 5% of paprika of different colors. An increase in the content of red paprika juice significantly decreased the lightness (CIE L*) of the yogurt, while the redness (CIE a*) increased significantly ($p < 0.05$). In particular, the redness of the yogurt with 2.5 and 5% red paprika juice was 6.89 and 11.19, respectively, values that were significantly higher than those for yogurt containing orange and yellow paprika ($p < 0.05$). The yellowness of the yogurt with 5% red paprika juice was similar to that of the yogurt without paprika juice. The redness and yellowness of the orange paprika yogurt were higher than those of the yogurt without the orange paprika juice but lightness was lower. In most yogurts containing paprika, the redness and yellowness increased and the lightness decreased as the amount of paprika juice was increased. According to Kim et al. (2016), the total carotenoid content of non-soil-cultivated paprika depends on its color: 55.80 mg/100 g dry weight for red, 62.57 mg/100 g dry weight for orange, and 35.32 mg/100 g dry weight for yellow paprika.

Total polyphenol content (TPC)

The TPC of the control yogurt was 132 μg/mL and 131 μg/mL (water extract and methanol extract, respectively) (Fig. 2). The TPC of the yogurt with the added paprika juice was higher in the water extract (159–287 μg/mL) than in the methanol extract (134–145 μg/mL). In contrast, Kang et al. (2018) found that the TPC of the methanol extracts was higher than that of the water extracts of yogurts containing red and green paprika. Addition of paprika significantly increased the TPC of the yogurt because paprika contains abundant polyphenols (Kang et al., 2018). Yogurt with added berries or aronia juice, known to be good sources of polyphenolic compounds, had higher TPC levels than plain yogurt (Nguyen and Hwang, 2016; Raikos et al., 2019).

Vitamin A and C levels in the paprika yogurt

The vitamin A content of the yogurt without paprika juice was 76.54 μg/100 mL, while that of the yogurt with paprika juice was 111.15–161.06 μg/100 mL (Fig. 3). Vitamin A content in the yogurts containing 5% red and orange paprika was significantly higher than that in the yogurt containing 5% yellow paprika ($p < 0.05$). Similarly, vitamin C levels were 610–1920 μg/100 mL for the red paprika yogurt, 640–2,270 μg/100 mL for the orange paprika yogurt, and 100–1,360 μg/100 mL for the yellow paprika yogurt. The yellow paprika yogurt had the lowest vitamin C levels. In agreement, Chávez-Mendoza et al. (2015) observed a lower vitamin C level in yellow paprika than red, orange, or green paprika. In contrast, Nerdy (2018) observed that the vitamin C content in the yellow, orange, and red paprika was 159.61, 121.38, and 81.19 mg/100 g, respectively. Thus, it can be inferred that paprika-containing yogurt has abundant levels of vitamins A and C, which are good antioxidants.

Antioxidant activity

The antioxidant activities of both the water and methanol extracts of the yogurts were significantly increased with the addition of paprika of different colors ($p < 0.05$) (Fig. 4). Interestingly, the antioxidant activities of the methanol extracts of the yogurts with paprika of different colors were 2 to 4-fold higher than those of the water extracts, although the TPC values of the water extracts were higher than those of the methanol extracts. This is probably because other bioactive compounds in the methanol extracts may mainly act on the antioxidant activities. Fresh peppers contain large amounts of phenolic compounds and vitamin C, and their various colors are because of the different carotenoid pigments, including β-carotene, with pro-vitamin A activity, and carotenoids such as capsanthin, capsorubin, and cryptocapsin, which are effective at scavenging free radicals (Deepa et al., 2006; Chávez-Mendoza et al., 2015).

Changes in the characteristics of the paprika yogurt during storage

Since the antioxidant activity and peptide content of yogurt with orange paprika juice were shown to be higher than the yogurts with other color paprika and there was little difference between the added levels, yogurt with 2.5% orange paprika juice was selected to further evaluate its physiochemical characteristics during a storage period of 15 d at 4°C (Table 3, Fig. 5).

pH and TA

At the end of the storage period, the pH of the control and paprika yogurt decreased significantly (4.47 and 4.46, respectively; $p < 0.05$). The pH of the paprika yogurt was 4.46–4.64, values that were lower than the pH of the control (4.47–4.76). During the storage period, the pH of yogurt was 4.46–4.76, while that of the semisolid type yogurt sold in Korea is 4.18–4.60 (Won et al., 2018). The pH of the yogurt is determined by the organic acid produced during fermentation. The pH range for improving the functionality and flavor is 4.1–4.7 (Won et al., 2018; Lee et al., 2017). Therefore, the paprika addition to yogurt helps maintain a suitable pH during storage.
In addition, TA, a parameter that relates to yogurt quality, increased in both the control and paprika yogurts with the length of the storage. The TA of the control was 0.77% on day 1 and 0.97% on day 15, while that of the paprika yogurt was 0.83% on day 1 and 1.01% on day 15. The TA of the control and paprika yogurt on day 15 of storage was significantly higher than that on day 1 of storage ($p < 0.05$). A TA for yogurt of 0.85–1.20% is suitable for Korean consumers (Lee et al., 2006). The best quality yogurt is when the TA is 1.0-1.1% (Kim et al., 1993). Therefore, the results obtained in this study indicate that the paprika yogurt was well preserved over 15 days of storage.

LAB count

The LAB count of the control and paprika yogurt did not change during storage. As the storage time increased, the viscosity of control and paprika yogurt increased significantly ($p < 0.05$). However, there was little change in the amino acid content; this may be because of poor hydrolysis of proteins and unchanged LAB counts during storage.

Syneresis

Syneresis of the paprika yogurt increased with storage time. Layer separation occurred during the storage period even though 0.2% pectin was added to help prevent layer separation of the yogurt. This increased syneresis is believed to have resulted in an increased viscosity of the yogurt during the storage period.

Color values

The CIE L*, a*, and b* values of the control and the paprika yogurt did not change over the 15 days, but the CIE b* value was significantly higher in the paprika yogurt than in the control, believed to be because of the addition of orange paprika juice.

TPC and antioxidant activity

The TPC of the paprika yogurt (157–163 μg/mL for water extracts and 114–146 μg/mL for methanol extracts) was slightly higher than that of the control yogurt (140–146 μg/mL for water extracts and 114–134 μg/mL for methanol extracts) (Fig. 5A and B). For both the control and paprika yogurts, the antioxidant activities of the methanol extracts did not change but those of the water extracts increased significantly ($p < 0.05$ Fig. 5C and D). In addition, antioxidant activities of the water and methanol extracts of the paprika yogurt were higher than those of the control yogurt. These results show that the addition of paprika juice increased TPC and antioxidant activities. A significant positive correlation between TPC and antioxidant activities in bell peppers has been reported (Kim et al., 2011; Chávez-Mendoza et al., 2015; Zhuang et al., 2012; Medina-Juárez et al., 2012).

Conclusion

LAB growth in yogurt was not affected by addition of different colored paprika juices. Furthermore, the addition of paprika juices increased TPC, levels of vitamin A and C, and antioxidant activity of yogurt. Thus, this study suggests that paprika can be a good natural food additive for the development of functional yogurts that can have an enhanced antioxidant effect and can be used as a natural pigment to enhance the visual effects of yogurt using a variety of paprika colors.

Conflict of interest

The authors declare no potential conflict of interest.

Acknowledgments

We thank Ju Hee Kim and Woong Lae Kim for their valuable contribution in the analysis of experiments.

Ethics Approval

This study did not require IRB/IACUC approval because there are no human and animal participants.

References

Abeysinghe DC, Li X, Sun C, Zhang W, Zhou C, Chen K. 2007. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. Food Chem 104:1338-1344.

Apostolidis E, Kwon YI, Shetty K. 2007. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol 8:46-54.

Arimboor R, Natarajan RB, Menon KR, Chandrasekhar LP, Moorkoth V. 2015. Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: analysis and stability—a review. J Food Sci Techol 52:1258-1271.

Arioui F, Saada DA, Cheriguene A. 2017. Physicochemical and sensory quality of yogurt incorporated with pectin from peel of *Citrus sinensis.* Food Sci Nutr 5: 358-364.

Bernaert N, Wouters D, De Vuyst L, De Paepe D, De Clercq H, Van Bockstaele E, De Loose M, Van Droogenbroeck B. 2013. Antioxidant changes of leek (*Allium ampeloprasum* var. *porrum*) during spontaneous fermentation of the white shaft and green leaves. J Sci Food Agric 93:2146–2153.

Chávez-Mendoza C, Sanchez E, Muñoz-Marquez E, Sida-Arreola JP, Flores-Cordova MA. 2015. Bioactive Compounds and Antioxidant Activity in Different Grafted Varieties of Bell Pepper. Antioxidants 4: 427-446.

Chen L, Kang YH. 2013. Anti-inflammatory and antioxidant activities of red pepper (*Capsicum annuum* L.) stalk extracts: Comparison of pericarp and placenta extracts. J Funct Foods 5:1724-1731.

Deepa N, Kaur C, Singh B, Kapoor HC. 2006. Antioxidant activity in some red sweet pepper cultivars. J Food Compos Anal 19: 572-578.

Di Cagno R, Coda R, De Angelis M, Gobbetti M. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 33:1-10.

El-Abbadi NH, Dao MC, Meydani N. 2014. Yogurt: role in health and active aging. Am J Clin Nutr 99:1263S-70S.

Food Hygiene Law Compilation Conference. 2001. The Korea Food Hygiene Regulations. Jigu Publishing Co. Seoul, Korea. pp 314-315.

Goodno CC, Swaisgood HE, Catignani GL. 1981. A fluorimetric assay for available lysine in proteins. Anal Biochem 115:203-211.

Halah MF, Nayra SM. 2011. Use of natural plant antioxidant and probiotic in the production of novel yogurt. J Evol Biol Res 3:12-18.

Hassan NM, Yusof NA, Yahaya AF, Rozali NNM, Othman R. 2019. Carotenoids of *Capsicum* Fruits: Pigment Profile and Health-Promoting Functional Attributes. Antioxidants 8:469 https://doi.org/10.3390/antiox8100469.

Jovanovic-Malinovska R, Kuzmanova S, Winkelhausen E. 2014. Oligosaccharide Profile in Fruits and Vegetables as Sources of Prebiotics and Functional Foods. Int J Food Prop 17: 949-965.

Kang SH, Yu MS, Kim JM, Park SK, Lee CH, Lee HG, Kim SK. 2018. Biochemical, Microbiological, and Sensory Characteristics of Stirred Yogurt Containing Red or Green Pepper (Capsicum annuum cv. Chungyang) Juice. Korean J Food Sci Anim Resour 38:451-467.

Kantar MB, Anderson JE, Lucht SA, Mercer K, Bernau V, Case KA, Le NC, Frederiksen MK, DeKeyser HC, Wong ZZ, Hastings JC, Baumler DJ. 2016. Vitamin Variation in Capsicum Spp. Provides Opportunities to Improve Nutritional Value of Human Diets. PLoS One 11:e0161464. Doi:10.1371/journal.pone.0161464.

Kim JS, Ahn J, Lee SL, Moon BK, Ha TY, Kim S. 2011. Phytochemicals and Antioxidant Activity of Fruits and Leaves of Paprika (*Capsicum Annuum* L.,var. *Special*) Cultivated in Korea. J Food Sci 76:C193-C198.

Kim JS, An CG, Park JS, Lim YP, Kim S. 2016. Carotenoid profiling from 27 types of paprika (*Capsicum annuum* L.) with different colors, shape, and cultivation methods. Food Chem 201:64-71.

Kim MS, Ahn ES, Shin DH. 1993. Physico-chemical properties of commercial yoghurt in Korea. Korean J Food Sci Technol 25:340-344.

Lee HJ, Pak HO, Lee JM. 2006. Fermentation properties of yogurt added with rice bran. Korean J Food Cookery Sci 22:488-494.

Lee JH, Park HY, Won JI, Park HI, Choi ID, Lee SK, Park JY, Joe DH, Jeon YH, Oh SK, Han SI, Choi HS. 2017. Quality characteristics of commercial liquid type yogurt in Korea. Korean J Food Preserv 24:865-870.

Lee Wj, Lucey JA. 2010. Formation and Physical Properties of Yogurt. Asian-Australas J Anim Sci 23:1127-1136.

Lorusso A, Coda R, Montemurro M, Rizzello CG. 2018. Use of Selected Lactic Acid Bacteria and Quinoa Flour for Manufacturing Novel Yogurt-Like Beverages. Foods 7(4). pii: E51. doi: 10.3390/foods7040051.

Madadlou A, Mousavi ME, Emam-djomeh Z, Ehsani MR, Sheehan D. 2009. Sonodisruption of Re-Assembled Casein Micelles at Different pH Values. Ultrasonics Sonochemistry 16: 644–648.

Maeda H, Saito S, Nakamura N, Maoka T. 2013. Paprika Pigments Attenuate Obesity-Induced Inflammation in 3T3-L1 Adipocytes. ISRN Inflamm 2013:763758. doi: 10.1155/2013/763758.

Marín A, Ferreres F, Tomás-Barberán FA, Gil MI. 2004. Characterization and quantitation of antioxidant constituents of sweet pepper (Capsicum annuum L.). J Agric Food Chem 52:3861-3869.

Medina- Juárez LA, Molina-Quijada DMA, del Toro SCL, González-Aguilar GA, Gámez-Meza N. 2012. Antioxidant activity of peppers (*Capsicum annuum* L) extracts and characterization of their phenolic constituents. Interciencia 3:588–593.

Nerdy N. 2018. Determination of Vitamin C in Various Colours of Bell Pepper (Capsicum annuum L.) by Titration Method. ALCHEMY Jurnal Penelitian Kimia 14:164-177.

Nguyen L, Hwang ES. 2016. Quality Characteristics and Antioxidant Activity of Yogurt Supplemented with Aronia (*Aronia melanocarpa*) Juice. Pre Nutr Food Sci 21:330-337.

Raikos V, Ni H, Hayes H, Ranawana V. 2019. Antioxidant Properties of a Yogurt Beverage Enriched with Salal (Gaultheria shallon) Berries and Blackcurrant (Ribes nigrum) Pomace during Cold Storage. Beverages doi:10.3390/beverages5010002.

Saccaro DM, Hirota CY, Tamie AY, de Oliveira MN. 2011. Evaluation of different selective media for enumeration of probiotic micro-organisms in combination with yogurt starter cultures in fermented milk. Afr J Microbiol Res 5:3901-3906.

Šaponjac VT, Četojević-Simin D, Ćetković G, Čanadanović-Brunet J, Djilas S, Mandić A, Tepić A. 2014. Effect of extraction conditions of paprika oleoresins on their free radical scavenging and anticancer activity. Cent Eur J Chem 12:377-385.

Savaiano DA. 2014. Lactose digestion from yogurt: mechanism and relevance. Am J Clin Nutr 99:1251S-5S.

Schlesinger WH, Bernhardt ES. Biochemistry; An Analysis of Global Change. 3rd ed. Elsevier p.259 ISBN 978-0-12-385874-0 Waltham MA, USA 2013.

Sinaga H, Bansal N, Bhandari B. 2017. Effects of milk pH alteration on casein micelle size and gelation properties of milk. Int J Food Prop 20:179-197.

Tsui PF, Lin CS, Ho LJ, Lai JH. 2018. Spices and Atherosclerosis. Nutrients 10:1724. doi: 10.3390/nu10111724.

U.S. Department of Agriculture. FoodData Central. Available from: https://fdc.nal.usda.gov/fdc-app.html#/food-details/342633/nutrients. Survey (FNDDS). FDC Published April 1, 2019.

Wang H, Livingston KA, Fox CS, Meigs JB, Jacques PF. 2013. Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. Nutr Res 33:18-26.

Wasilewska E, Zotkowska D, Wroblewska. 2019. Yogurt starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ameliorate symptoms and modulate the immune response in a mouse model of dextran sulfate sodium-induced colitis. J Dairy Sci 102:37-53

Wei X, Luo M, Xu L, Zhang Y, Lin X, Kong P, Liu H. 2011. Production of fibrinolytic enzyme from Bacillus amyloliquefaciens by fermentation of chickpeas, with the evaluation of the anticoagulant and antioxidant properties of chickpeas. J Agric Food Chem 59:3957-3963.

Won JI, Lee JH, Park HI, Cho YU, Choi ID, Lee SK, Park HY, Park JY, Oh SK, Han SI, Choi HS. 2018. Quality characteristics of commercial semisolid type yogurt in Korea. J Korean Soc Food Sci Nutr 47:1185-1190.

Yu MS, Kim JM, Lee CH, Son YJ, Kim SK. 2014. Quality characteristics of stirred yogurt added with fermented red pepper. Korean J Food Sci An 34:408-414.

Zhuang Y, Chen L, Sun L, Cao J. 2012. Bioactive characteristics and antioxidant activities of nine peppers. J Funct Foods 4:331–338.

Ziarno M, Zaręba D. 2019. The effect of the addition of microbial transglutaminase before the fermentation process on the quality characteristics of three types of yogurt. Food Sci Biotechnol doi:10.1007/s10068-019-00640-6.

Table 1. Changes in pH, fermentation rate, titratable acidity, and viable cell count

Attributes	Treatment	Addition of paprika juice (%)		
		0	2.5	5
pH	Red	4.53±0.00	4.51±0.00^{A}	4.45±0.01^{B}
	Orange	4.53±0.00	4.49±0.00^{B}	4.46±0.00^{A}
	Yellow	4.53±0.00^{a}	4.47±0.01^{abC}	4.44±0.00^{bB}
Fermentation rate(pH/h)	Red	-0.48±0.01	-0.43±0.35	-0.45±0.02
	Orange	-0.48±0.01	-0.47±0.01	-0.47±0.01
	Yellow	-0.48±0.01	-0.46±0.00	-0.46±0.01
Titratable acidity(%)	Red	0.72±0.00^{c}	0.88±0.00^{b}	0.95±0.01^{aA}
	Orange	0.72±0.00^{c}	0.88±0.01^{b}	0.89±0.01^{aB}
	Yellow	0.72±0.00^{c}	0.88±0.01^{b}	0.90±0.01^{aB}
Viable cells, Log$_{10}$(CFU/g)	Red	10.35±0.07^{b}	10.45±0.07^{ab}	10.60±0.00^{a}
	Orange	10.30±0.14^{b}	10.50±0.14^{ab}	10.85±0.07^{a}
	Yellow	10.40±0.00	10.60±0.14	10.55±0.21

Values are mean±standard deviation (n=3)

Different small letters in the same row and capitalized letter in the same column indicated significant difference (p<0.05).

Table 2. Change in the color of paprika yogurt according to addition of paprika juice

Color value	Treatment	Addition of paprika juice (%)		
		0	2.5	5
CIE L* (Lightness)	Red	90.41±0.04^{a}	84.14±0.04^{bC}	80.71±0.04^{cC}
	Orange	90.41±0.04^{a}	86.40±0.25^{bB}	86.56±0.02^{bB}
	Yellow	90.41±0.04^{a}	91.13±0.02^{aA}	90.42±0.07^{bA}
CIE a* (Redness)	Red	-4.29±0.02^{c}	6.89±0.20^{bA}	11.19±0.54^{aA}
	Orange	-4.29±0.02^{c}	-2.42±0.06^{bB}	0.98±0.02^{aB}
	Yellow	-4.29±0.02^{c}	-4.92±0.06^{bC}	-5.38±0.10^{cC}
CIE b* (Yellowness)	Red	19.27±0.05^{c}	15.28±0.20^{bB}	18.36±0.23^{aB}
	Orange	19.27±0.05^{c}	17.85±0.53^{bA}	25.64±0.03^{aA}
	Yellow	19.27±0.05^{c}	13.94±0.05^{bC}	16.97±0.35^{aC}

Values are mean±standard deviation (n=3)

Different small letters in the same row and capitalized letter in the same column indicated significant difference (p<0.05).

Table 3. Changes in pH, titratable acidity, viable cell count, viscosity, peptide concentration, and syneresis during storage

Treatment		Storage period (Days) 1	5	10	15
pH	Control	4.76±0.02^{a}	4.66±0.02^{b}	4.53±0.00^{c}	4.47±0.04^{d}
	Orange[1)]	4.64±0.02^{a}	4.65±0.01^{a}	4.48±0.01^{b}	4.46±0.02^{b}
Titratable acidity(%)	Control	0.77±0.01^{d}	0.87±0.00^{c}	0.94±0.02^{b}	0.97±0.11^{a}
	Orange	0.83±0.01^{d}	0.90±0.02^{c}	0.98±0.02^{b}	1.01±0.03^{a}
Viable cell(CFU/mL)	Control	10.17±0.12^{b}	10.20±0.10^{b}	10.60±0.15^{a}	10.60±0.00^{a}
	Orange	10.23±0.12^{c}	10.20±0.02^{c}	10.37±0.06^{a}	10.36±0.23^{a}
Viscosity(cps)	Control	20689±1361^{c}	44933±10399^{b}	57367±4983^{a}	66689±20367^{a}
	Orange	29822±8664^{c}	48956± 4721^{b}	53956±8833^{b}	73756±21977^{a}
Peptide concentration (mg/ml)	Control	0.21±0.00	0.21±0.00	0.21±0.00	0.21±0.00
	Orange	0.33±0.00	0.33±0.00	0.33±0.00	0.33±0.00
Syneresis(%)	Control	29.63±3.86^{b}	31.18±1.40^{b}	37.39±1.01^{a}	38.05±1.26^{a}
	Orange	33.63±2.95^{b}	36.25±0.95^{ab}	36.85±2.66^{ab}	40.04±0.64^{a}
Color CIE L* (Lightness)	Control	89.57±1.24^{a}	90.22±0.58^{a}	86.90±0.48^{b}	88.16±0.35^{b}
	Orange	87.59±1.16	86.01±0.91	86.35±0.13	87.22±0.68
Color CIE a* (Redness)	Control	-4.37±0.50^{b}	-4.57±0.17^{c}	-3.35±0.19^{a}	-3.96±0.16^{b}
	Orange	-2.51±0.50	-2.84±0.05	-2.80±0.02	-2.55±0.26
Color CIE b* (Yellowness)	Control	9.04±1.69^{a}	8.71±0.85^{a}	6.19±0.45^{b}	7.74±0.42^{a}
	Orange	17.49±1.92^{a}	14.53±1.43^{b}	14.74±0.19^{b}	15.98±1.08^{a}

[1)] Yogurt added with 2.5% orange paprika juice

Values are mean±standard deviation (n=3)

Different small letters in the same row and capitalized letter in the same column indicated significant difference (p<0.05).

Figure legends

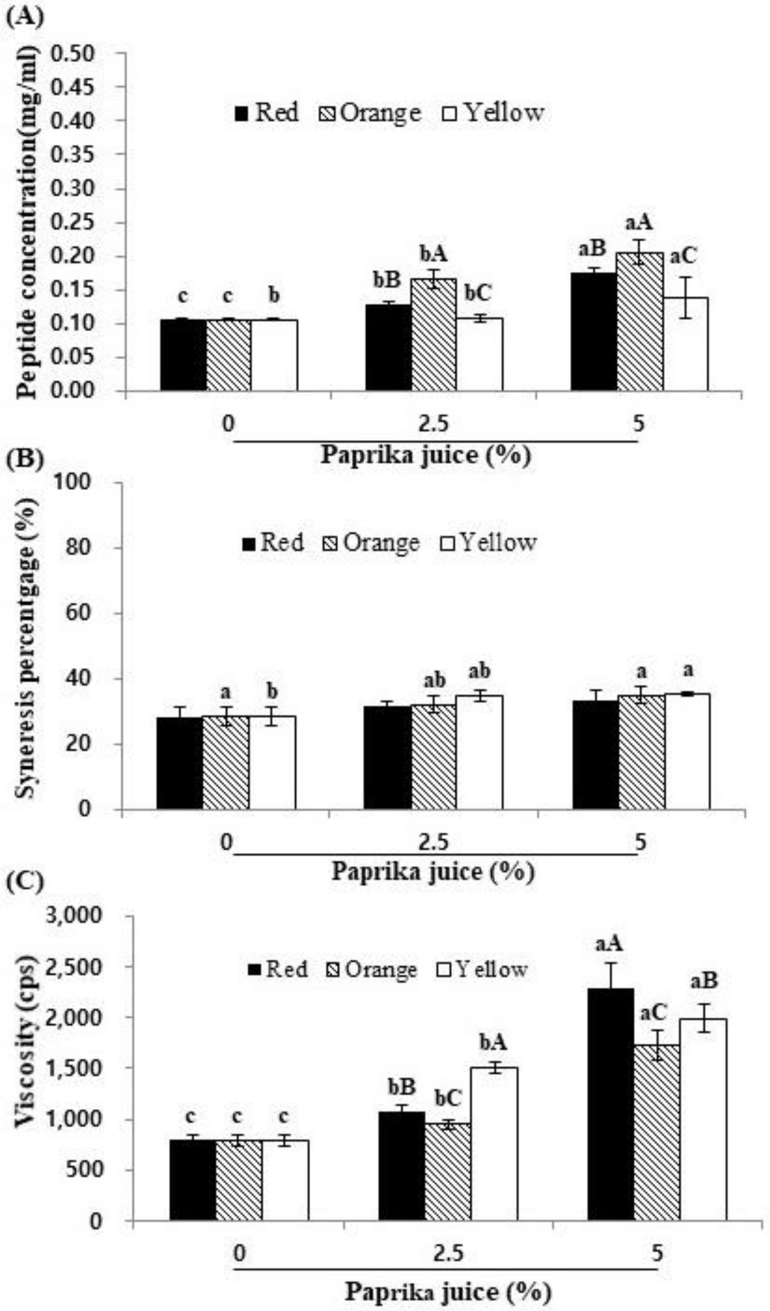

Fig. 1. Changes in peptide concentration, syneresis, and viscosity of paprika yogurt by the addition of different colored paprika juices. (A) Peptide concentration, (B) Syneresis, (C) Viscosity. Different capitalized letters indicate significant differences at the same concentration of paprika juice, regardless of paprika color, and small letters indicate significant differences at the different concentration of paprika juice in the same color; p<0.05.

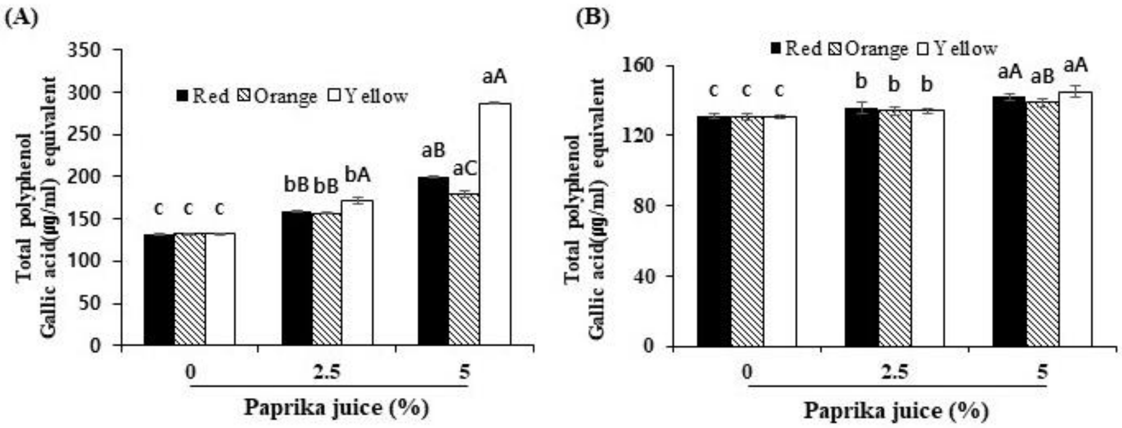

Fig. 2. Total polyphenol content in extracts of yogurt containing different levels of paprika juices. Total polyphenol content in the (A) water extracts and (B) 95% methanol extracts. Different capitalized letters indicate significant differences at the same concentration of paprika juice, regardless of paprika color, and small letters indicate significant differences at the different concentration of paprika juice in the same color; $p<0.05$.

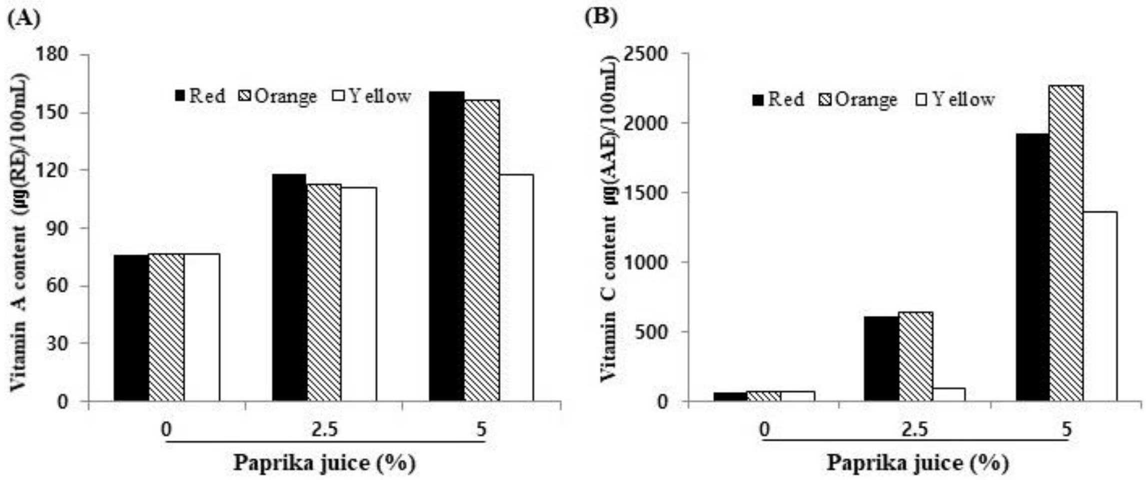

Fig. 3. Vitamin A and C levels in yogurt containing paprika of different colors. (A) Vitamin A and (B) Vitamin C levels.

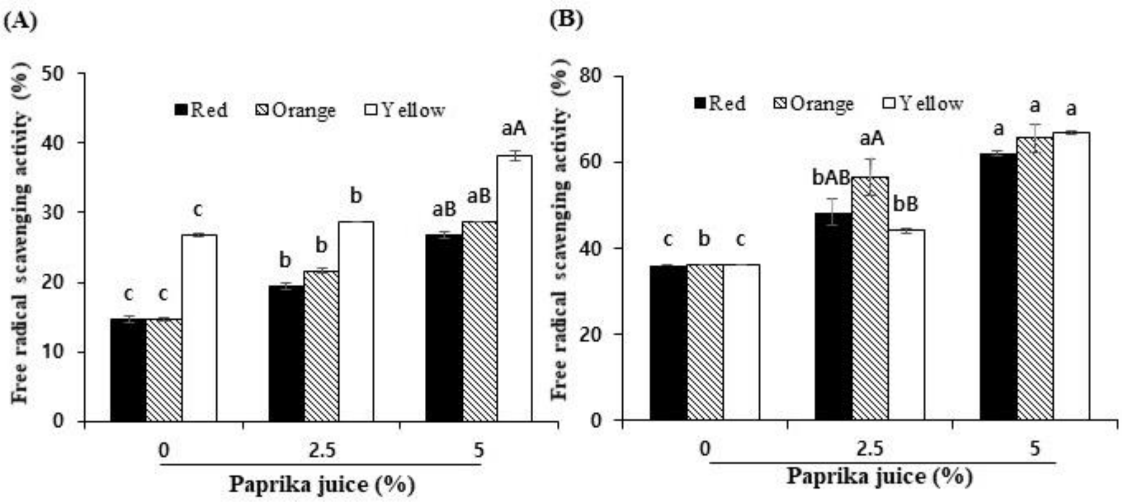

Fig. 4. Antioxidant activity of extracts of yogurt containing different levels of paprika juices. Antioxidant activity of the (A) water extracts and (B) 95% methanol extracts. Different capitalized letters indicate significant differences at the same concentration of paprika juice, regardless of paprika color, and small letters indicate significant differences at the different concentration of paprika juice in the same color; p<0.05.

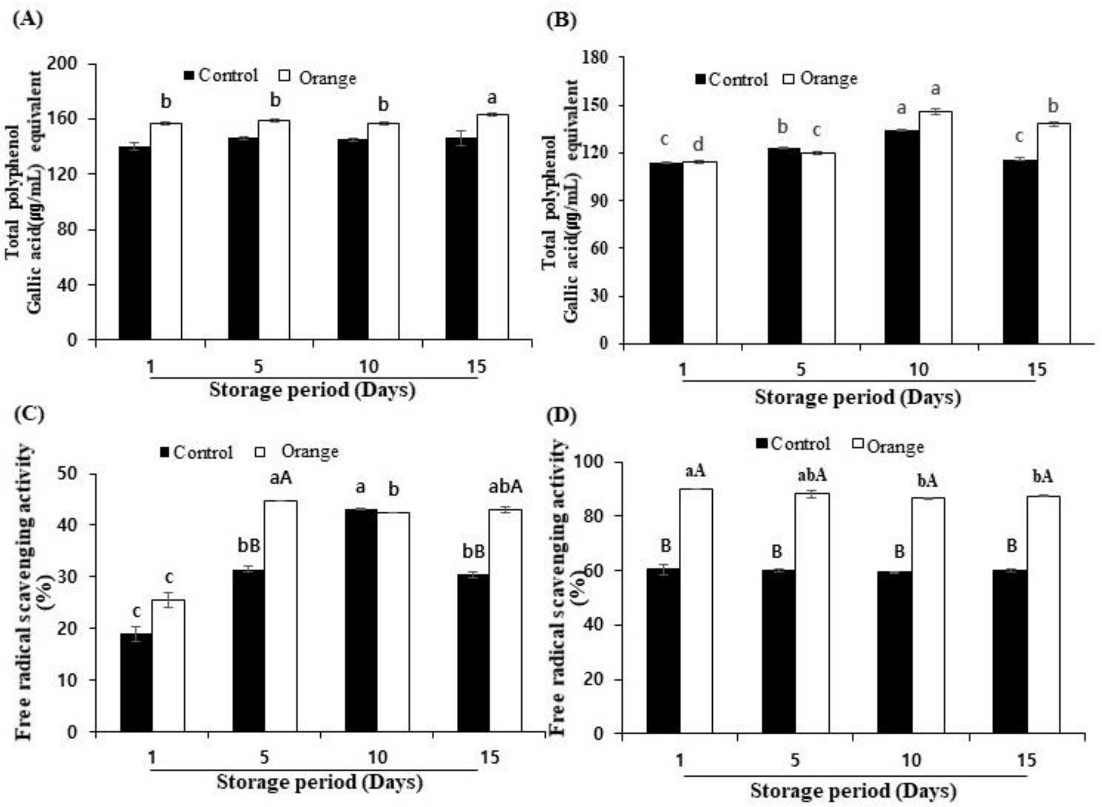

Fig. 5. Effect of 15-day storage at 4°C on total polyphenol content and antioxidant activity of yogurt containing 2.5% orange paprika juice. Change in the total polyphenol content of (A) water and (B) methanol extracts. Change in the antioxidant activity of (C) water and (D) methanol extracts. Different capitalized letters indicate significant difference between control and orange paprika yogurt, and small letters indicate significant difference in control or orange paprika yogurt (*p*<0.05).