

## ARTICLE

# Antioxidant Activity and Quality Characteristics of Yogurt Added Green Olive Powder during Storage

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## Abstract

The objective of this study was to determine the antioxidant and quality characteristics of yogurt added green olive powder stored at 4°C for 15 d. The following four groups were used in this study: Control group (GY0), Yogurt added with 1% green olive (GY1), with 3% green olive (GY3), and with 5% green olive (GY5). The more time of titratable acidity went by, the more it increased. Except GY0, viscosity tended to decrease in other groups ( $p > 0.05$ ), and the more time of syneresis went by, the more it increased, but GY3 of them showed the lowest syneresis. Lactic acid bacteria showed no significant with GY0 until 5 d, but after that, GY1, GY3 and GY5 showed lower than GY0. Yogurt added green olive showed darker color than GY0 (low  $L^*$  and high  $a^*$ ). The antioxidant activity of GY5 was found to be the highest among the four groups at day 1 of storage. Total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and reducing power of GY5 was found to be the highest among the four groups at day 1 of storage which were 6.96 mg GAE/kg, 47.53%, and 0.57, respectively. In the sensory evaluation sweet and overall of GY3 indicated the highest score among the four groups. Results of this study demonstrated that green olive powder might be used to improve the antioxidant capacity and sensory characteristics of yogurt.

**Keywords** yogurt, green olive powder, antioxidant activity, sensory evaluation, dairy

## Introduction

Yogurt is a probiotic dairy product generated by lactic acid bacteria through fermentation of milk. Yogurt can promote the reduction of blood cholesterol, absorption of vitamins and minerals, improvement of digestive tract, and absorption of digestion and lactose (Gilliland and Seck, 1977; MacGregor *et al.*, 2002). In addition, yogurt can inhibit colorectal cancer and diabetes mellitus. Therefore, yogurt has been expanded to prevent and cure diseases such as hyperlipidemia (Cho *et al.*, 2006; Goldin and Gorbach, 1984).

Recently, interest in natural food additives and integration of substance for enhancement of health has increased (Varga, 2006). Natural food materials have been added to yogurt to increase its functionality, including the addition of mulberry, green tea, white tea, and black tea (Muniandy *et al.*, 2016) wine grape pomace (Tseng and Zhao, 2013) and grape (Karaaslan *et al.*, 2011). Studies that manu-

facture yogurt with more intensified biological activity are progressing.

Olive oil and table olive are important components of Mediterranean diet. They are consumed all over the world (Pereira *et al.*, 2006) Olive is a fruit containing abundant antioxidant substance, including phenolic compounds such as verbascoside, ligstroside, and oleuropein (Ryan *et al.*, 1999) of these antioxidants, oleuropein has been found in Olive (*Olea europaea*) leaves and raw olive (Bianco and Uccella, 2000; Soler-rivas *et al.*, 2000). In many *in vivo* and *in vitro* studies, oleuropein has shown various biological activities, including antioxidant and antimicrobial effect (Bisignano *et al.*, 1999; Speroni *et al.*, 1998). According to scientific view of European Food Safety Authority (2011), oleuropein can lower LDL cholesterol (LDL-C) level. However, no study has reported the antioxidant and quality characteristics of yogurt added with green olive powder. Therefore, the objective of this study was to determine the antioxidant and quality characteristics of yogurt added with green olive powder in shelf-life.

## Materials and Methods

### Preparation of green olive powder

Green olives were used as salted green olives imported from Spain. First, to remove saltiness of green olive, green olives were took out after soaking in water for 6 h. They were then freeze-dried using a freeze dryer (Ilshin Lab, MCFD 8510, Korea). Dried green olives were ground for 2-3 min with a grinder. Green olive powder was kept at -80°C in a deep freezer until use.

### Starter cultures

Lactic acid bacteria including *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactoba-*

*cillus acidophilus*, and *Bifidobacterium animalis* ssp. *lactis* were used as yogurt fermentation starter (Lyofast YAB 450 AB, Sacco srl., Italy).

### Yogurt preparation

Skim milk powder, pectin, white sugar, and green olive powder at 0%, 1%, 3%, and 5% were homogenized for 5 min using a homogenizer (Table 1). The mixture was sterilized at 85°C for 30 min followed by cooling to 42°C in a water bath. Yogurt culture was inoculated to the mixture and fermented at 37°C for 8 h until pH reached 4.5. After fermentation, all yogurt samples were stored at 4°C in a fridge. Samples were collected at interval of 5 d for 15 d for analysis (1, 5, 10 and 15 d).

### pH and titratable acidity

pH was determined using a pH meter (pH 900, Precisa Co., Switzerland). Titratable acidity was determined for all groups through neutralization titration until pH reached 8.3 using distilled water (3 g of stored yogurt sample in 27 mL of water). Then 0.1 N NaOH was used to calculate the amount of lactic acid (%) using the following equation:

$$LA\% = \frac{10 \times V_{NaOH} \times 0.009 \times 0.1}{W} \times 100$$

10 = Dilution factor;

W = Weight of sample (g) for titration;

$V_{NaOH}$  = Volume of NaOH used to neutralize the lactic acid;

0.1 = Normality of NaOH.

### Viscosity and syneresis

Viscosity of yogurt sample stored at 4°C was measured using a viscometer (Model LVDV-E, Brookfield Engineering Lab. Inc., USA). Samples were stirred for 5 min

**Table 1. Normal compositions of yogurt added with green olive powders**

| Ingredients        | Treatments |       |        |       |        |       |        |       |
|--------------------|------------|-------|--------|-------|--------|-------|--------|-------|
|                    | GY0        |       | GY1    |       | GY3    |       | GY5    |       |
|                    | g (mL)     | %     | g (mL) | %     | g (mL) | %     | g (mL) | %     |
| Green olive powder | 0          | 0     | 10.1   | 0.99  | 30.3   | 2.96  | 50.5   | 4.94  |
| Milk               | 850        | 83.98 | 850    | 83.15 | 850    | 83.45 | 850    | 79.82 |
| Powdered skim milk | 40         | 3.95  | 40     | 3.91  | 40     | 3.88  | 40     | 3.76  |
| Sugar              | 15         | 1.48  | 15     | 1.47  | 15     | 1.46  | 15     | 1.41  |
| Pectin             | 2          | 0.20  | 2      | 0.20  | 2      | 0.19  | 2      | 0.19  |
| Distilled water    | 105        | 10.37 | 105    | 10.27 | 105    | 10.19 | 105    | 9.86  |
| Starter            | 0.2        | 0.02  | 0.2    | 0.02  | 0.2    | 0.02  | 0.2    | 0.02  |
| Total              | 1012.2     | 100   | 1022.3 | 100   | 1042.5 | 100   | 1062.7 | 100   |

GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

before measurement. Then, all viscosity values were measured at 50 rpm with spindle No.63.

Yogurt syneresis (release of whey) was determined using the centrifugation method described by Keogh and O' Kennedy with some modifications (1998). Briefly, yogurt (30 g) was centrifuged (640×g, 20 min, 4°C). The clear supernatant was harvested and weighed. Syneresis was calculated using the following equation (Keogh and O' Kennedy, 1998):

$$\text{Syneresis} = \frac{\text{weight of supernatant (g)}}{\text{weight of yogurt sample (g)}} \times 100\%$$

### Lactic acid bacteria

The cell viability of lactic acid bacteria (LAB) was measured using the spread plate method with MRS agar (Oxoid Ltd.) at 37°C for 24 h. Samples (100 µL) were added to 900 µL of 0.85% sterile saline and sequentially diluted 10 times with 0.85% saline, then 100 µL of each dilution was spread on plates, followed by incubation at 37°C for 24 h. Total viable cell numbers were expressed as log values (Park and Oh, 2005).

### Color measurement

Color was checked using a colorimeter (NR-300, Nippon Denshoku, Japan). The instrument was calibrated before measurements using standard white plate supplied with the instrument. Lightness (CIE L\*), redness (CIE a\*), and yellowness (CIE b\*) values were measured. Measurements were repeated three times for each treatment group.

### Total polyphenol content (TPC) and antioxidant activity

Total phenolic content was determined using the method of Wei (2011) with slight modification. Briefly, after mixing 100 µL of test solution and 100 µL of Folin-Caltheous phenol reagent 1 N solution, the mixture was allowed to react at room temperature for 3 min. Then 300 µL of 1 N Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture followed by incubation at room temperature for 90 min. Then 1 mL of distilled water was added to the mixture to complete the reaction. After that, the absorbance of the mixture was measured at wavelength of 725 nm using a spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Korea). Results were expressed as mg of gallic acid equivalents (GAEs) per 10 g of sample using gallic acid as the standard.

The reducing power of tannic acid was determined using the method of Oyaizu (1986) with slight modifica-

tion (Gülçin, 2006). Briefly, tannic acid in 1 mL of distilled water at different concentrations (15–45 µg/mL) was mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Aliquot (2.5 mL) of trichloroacetic acid (10%) was added to the mixture. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl<sub>3</sub> (0.5 mL, 0.1%). After that, the absorbance of the mixture was measured at wavelength of 700 nm using a spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Korea).

DPPH radical scavenging activity was determined using the method of Blois (1958). To determine the antioxidant activity of sample, DPPH radical scavenging activity ( $\alpha$ ,  $\alpha$ -Diphenyl- $\beta$ -picrylhydrazyl, sigma) was measured. Briefly, 1 mL of 1.5×10<sup>-4</sup> M DPPH radical scavenging activity solution was added to 4 mL of test solution and stirred. The mixture was then incubated at room temperature for 30 min. The absorbance of the mixture was then measured at wavelength of 517 nm using a spectrophotometer (OPTIZEN 2120UV, Mecasys Co., Ltd., Korea).

### Sensory evaluation

Sensory evaluation of GY0, GY1, GY3 and GY5 (stored at 4°C) was estimated by 30 trained panelists, 3 d after production. These panelists were members of staff and students at Konkuk University, Seoul, Korea. Each item evaluated was given a score of 7 points hedonic scale; like extremely = 7, like very much = 6, like moderately = 5, neither like nor dislike = 4, dislike moderately = 3, dislike very much = 2, dislike extremely = 1. The following items were evaluated: color, flavor, sweet, sour, texture, oily, and overall sensory characteristic. Descriptions about these items were given to panelists prior to evaluation.

### Statistical analysis

All data from three replicates were analyzed by one-way analysis of variance using SPSS/PC Statistics 18.0 software (SPSS Inc., USA). All data are presented as means with standard deviation. Duncan's multiple range tests were used to determine significance among means. Statistical significance was considered when *p*-value was less than 0.05 (*p*<0.05).

## Results and Discussion

### pH and Titratable acidity

Results of pH and titratable acidity value of yogurt

added green olive powder stored at 4°C for 15 d are shown in Table 2. The pH value of yogurt after finishing fermentation ranged from 4.38 to 4.41. After that, incipient pH of storage ranged from 4.44 to 4.55. The decrease of pH during the storage might be due to accumulation of lactic acid by metabolic activity of bacteria (Tseng and Zhao, 2013). The pH was decreased in all treatment groups except the GY5 group during the storage. It might be resulted from the number of lactic acid bacteria, and that of GY5 was fewer than other groups (Table 4) in every measured day. The conversion of lactic acid from lactose by lactic acid bacteria would be relatively decreased in GY5 than other groups due to its lower amount of lactic acid bacteria during storage and it result in constant pH in GY5.

According to Lee and Hwang (2006), the optimum pH of thick fermented milk coming into the market is from 3.27 to 4.59. In this study, after storing yogurt for 15 d at 4°C, the pH also fell into this optimum range. This mean that the quality of yogurt added green olive yogurt is not different from the quality of fermented milk in the market.

The initial titratable acidity values of yogurt ranged from

0.92 to 0.94%. After storage, titratable acidity values were increased in all treatment groups. After 15 d of storage, titratable acidity values ranged from 1.07 to 1.14%. Davis (1970) has reported that titratable acidity of normal product is 0.72 to 1.20%. Titratable acidity of yogurt samples in this study fell into this range.

### Viscosity and syneresis

Results of viscosity and syneresis of yogurt samples after storing at 4°C for 15 d are shown in Table 3. Experimental groups showed no significant changes in viscosity up to 5 d except for GY5 which showed significant reduction in viscosity. After 10 d of storage, viscosity of GY1 was not significantly changed compared to its initial value. After storage of five days, viscosity of GY1, GY3, and GY5 were higher than that of GY0 ( $p>0.05$ ). The more time went by, the more viscosity of GY5 decreased ( $p<0.05$ ), and GY3 decreased after 10 d significantly. GY1 didn't show significant difference through all storage duration, and GY0 showed a decreasing tendency after 5 d when comparing with 1 d.

This result was in agreement with the result of a previous study showing that yogurt added with yuza extract

**Table 2. pH and titratable acidity during the storage of yogurt added with green olive**

|                    | Storage period (d) | GY0                      | GY1                      | GY3                     | GY5                      |
|--------------------|--------------------|--------------------------|--------------------------|-------------------------|--------------------------|
| pH                 | 1                  | 4.55±0.02 <sup>Aa</sup>  | 4.53±0.02 <sup>Aa</sup>  | 4.45±0.02 <sup>Ab</sup> | 4.44±0.01 <sup>Ab</sup>  |
|                    | 5                  | 4.41±0.04 <sup>Bb</sup>  | 4.44±0.02 <sup>Bab</sup> | 4.47±0.01 <sup>Aa</sup> | 4.46±0.02 <sup>Aa</sup>  |
|                    | 10                 | 4.36±0.02 <sup>Cb</sup>  | 4.39±0.03 <sup>Cb</sup>  | 4.39±0.03 <sup>Bb</sup> | 4.44±0.02 <sup>Aa</sup>  |
|                    | 15                 | 4.37±0.01 <sup>BCb</sup> | 4.36±0.02 <sup>Cb</sup>  | 4.37±0.02 <sup>Bb</sup> | 4.46±0.02 <sup>Aa</sup>  |
| Titratable acidity | 1                  | 0.94±0.01 <sup>Da</sup>  | 0.92±0.00 <sup>Cb</sup>  | 0.93±0.00 <sup>Db</sup> | 0.93±0.01 <sup>Db</sup>  |
|                    | 5                  | 1.03±0.01 <sup>Ca</sup>  | 1.01±0.01 <sup>Bb</sup>  | 1.02±0.01 <sup>Cb</sup> | 1.03±0.01 <sup>Ca</sup>  |
|                    | 10                 | 1.08±0.01 <sup>Ba</sup>  | 1.06±0.01 <sup>Ab</sup>  | 1.07±0.01 <sup>Bb</sup> | 1.08±0.01 <sup>Bab</sup> |
|                    | 15                 | 1.13±0.00 <sup>Ab</sup>  | 1.07±0.00 <sup>Ad</sup>  | 1.10±0.00 <sup>Ac</sup> | 1.14±0.01 <sup>Aa</sup>  |

<sup>1</sup>GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

<sup>2</sup>Means with different superscripts (<sup>A-D</sup> in the same column and <sup>a-d</sup> in the same row) differ significantly ( $p<0.05$ ).

All values are means ± standard deviation for three replicates.

**Table 3. Viscosity and syneresis during the storage of yogurt added with green olive**

|           | Storage period (d) | GY0                      | GY1                      | GY3                      | GY5                      |
|-----------|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Viscosity | 1                  | 3490±336 <sup>Cc</sup>   | 5257±525 <sup>Ab</sup>   | 7957±784 <sup>Aa</sup>   | 8943±603 <sup>Aa</sup>   |
|           | 5                  | 5017±218 <sup>Ab</sup>   | 5380±735 <sup>Ab</sup>   | 7620±485 <sup>Aa</sup>   | 8063±31 <sup>Ba</sup>    |
|           | 10                 | 4593±304 <sup>ABa</sup>  | 4587±172 <sup>Aa</sup>   | 4755±448 <sup>Ba</sup>   | 4420±53 <sup>Ca</sup>    |
|           | 15                 | 4530±107 <sup>Ba</sup>   | 4535±197 <sup>Aa</sup>   | 4284±196 <sup>Ba</sup>   | 3875±260 <sup>Db</sup>   |
| Syneresis | 1                  | 23.02±3.13 <sup>Db</sup> | 25.55±5.15 <sup>Da</sup> | 14.28±3.18 <sup>Dc</sup> | 15.35±1.20 <sup>Dc</sup> |
|           | 5                  | 35.88±4.00 <sup>Ca</sup> | 34.08±2.98 <sup>Cb</sup> | 23.69±3.59 <sup>Cc</sup> | 24.21±3.48 <sup>Cc</sup> |
|           | 10                 | 61.25±7.14 <sup>Bb</sup> | 62.88±3.01 <sup>Ba</sup> | 49.53±4.65 <sup>Bd</sup> | 54.87±5.95 <sup>Bc</sup> |
|           | 15                 | 70.79±6.85 <sup>Ab</sup> | 69.16±4.00 <sup>Ab</sup> | 67.65±3.00 <sup>Ac</sup> | 69.82±4.37 <sup>Ab</sup> |

<sup>1</sup>GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

<sup>2</sup>Means with different superscripts (<sup>A-D</sup> in the same column and <sup>a-d</sup> in the same row) differ significantly ( $p<0.05$ ).

All values are means ± standard deviation for three replicates.

has higher viscosity than that of the control (Lee and Kim, 2008). Because green olive powder was added to yogurt during manufacturing of green olive yogurt, solid content was increased which might have increased the viscosity (Lee and Kim, 2008). Ramirez-Santiago *et al.* (2010) have reported that viscosity is increased when fiber is added to yogurt. Therefore, dietary fiber from table olive might have affected the viscosity of yogurt containing green olive powder as suggested by Jiménez *et al.* (2000).

Syneresis is directly affected by acidity and is inversely proportional to pH (Fox *et al.*, 2000). Results of syneresis of yogurt in this study revealed that the longer the storage duration, the higher the syneresis. When pH is lower (especially below 5.0), casein will approach to isoelectric point. Therefore, electrostatic repulsion is minimized because protein-to-protein interaction is promoted (Visser *et al.*, 1986; Marchesseau *et al.*, 1997). The ability of protein interacting with water and water-holding capacity of protein matrix will be decreased below pH 5.0 (Pastorino *et al.*, 2003).

### Lactic acid bacteria

Results of the number of lactic acid bacteria in yogurt

stored at 4°C for 15 d are shown in Table 4. The number of initial lactic acid bacteria in all groups was over 9.0 Log CFU/g. The number of lactic acid bacteria in yogurt after storage for 15 d was decreased to 7.7-8.7 Log CFU/g. Such number of lactic acid bacteria in all groups after 15 d of storage was high enough compared to standard yogurt. In intestinal canal, microbial count of probiotics for useful action is estimated to be at least 6 Log CFU/g (Akalin *et al.*, 2004). The number of total lactic acid bacteria of fermented drinks in Codex is regulated to be more than 7 Log CFU/g. However, when green olive powder is added to yogurt, lactic acid bacteria tend to be decreased more than control yogurt after 10 d of storage. Therefore, additional studies are needed to determine the antimicrobial action and the proper amount of green olive powder to be added to yogurt.

### Total polyphenol content (TPC) and antioxidant activity

Results of total polyphenol contents and antioxidant activity in yogurt after storage at 4°C for 15 d are shown in Table 5. TPC values in GY0, GY1, GY3, and GY5 were 4.30, 4.51, 5.85, and 6.96 mg GAE/kg, respectively

**Table 4. Lactic acid bacteria counts (Log CFU/g) during storage of yogurt added with green olive**

| Storage period (d) | GY0                     | GY1                      | GY3                      | GY5                     |
|--------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| 1                  | 9.38±0.80 <sup>Aa</sup> | 9.18±1.32 <sup>Aa</sup>  | 9.00±0.98 <sup>Aa</sup>  | 8.95±0.37 <sup>Aa</sup> |
| 5                  | 9.33±0.29 <sup>Aa</sup> | 9.13±0.53 <sup>Aa</sup>  | 8.95±1.37 <sup>Aa</sup>  | 8.94±0.57 <sup>Aa</sup> |
| 10                 | 9.15±0.12 <sup>Aa</sup> | 8.74±0.30 <sup>Aab</sup> | 8.64±0.17 <sup>Aab</sup> | 8.55±0.15 <sup>Ab</sup> |
| 15                 | 8.65±0.03 <sup>Ba</sup> | 7.82±0.24 <sup>Bb</sup>  | 7.72±0.02 <sup>Bb</sup>  | 7.69±0.20 <sup>Bb</sup> |

<sup>1</sup>GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

<sup>2</sup>Means with different superscripts (<sup>A,B</sup> in the same column and <sup>a,b</sup> in the same row) differ significantly ( $p < 0.05$ ).

All values are means ± standard deviation for three replicates.

**Table 5. Total polyphenol content (TPC) and antioxidant activity during storage of yogurt added with green olive**

|                       | Storage period (d) | GY0                      | GY1                      | GY3                       | GY5                      |
|-----------------------|--------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| TPC<br>(mg GAE/100 g) | 1                  | 4.30±0.17 <sup>Ac</sup>  | 4.52±0.26 <sup>Ac</sup>  | 5.85±0.30 <sup>Ab</sup>   | 6.96±0.13 <sup>Aa</sup>  |
|                       | 5                  | 4.05±0.26 <sup>Ab</sup>  | 4.42±0.27 <sup>Ab</sup>  | 5.13±0.60 <sup>ABab</sup> | 6.18±0.37 <sup>ABa</sup> |
|                       | 10                 | 4.02±0.17 <sup>Ac</sup>  | 4.23±0.20 <sup>Ac</sup>  | 5.08±0.10 <sup>ABb</sup>  | 6.00±0.16 <sup>ABa</sup> |
|                       | 15                 | 3.67±0.53 <sup>Ab</sup>  | 4.00±0.33 <sup>Ab</sup>  | 4.82±0.17 <sup>Bab</sup>  | 5.60±0.53 <sup>Ba</sup>  |
| Reducing power        | 1                  | 0.37±0.01 <sup>Ad</sup>  | 0.41±0.01 <sup>Ac</sup>  | 0.48±0.02 <sup>Ab</sup>   | 0.57±0.01 <sup>Aa</sup>  |
|                       | 5                  | 0.34±0.01 <sup>ABd</sup> | 0.39±0.01 <sup>ABc</sup> | 0.43±0.00 <sup>Bb</sup>   | 0.52±0.02 <sup>Ba</sup>  |
|                       | 10                 | 0.33±0.01 <sup>Bc</sup>  | 0.38±0.00 <sup>Bb</sup>  | 0.41±0.01 <sup>Ba</sup>   | 0.43±0.00 <sup>Ca</sup>  |
|                       | 15                 | 0.34±0.01 <sup>Bb</sup>  | 0.38±0.02 <sup>ABa</sup> | 0.40±0.01 <sup>Ba</sup>   | 0.40±0.01 <sup>Ca</sup>  |
| DPPH (%)              | 1                  | 40.13±2.22 <sup>Ab</sup> | 39.46±0.63 <sup>Ab</sup> | 44.84±1.28 <sup>Aa</sup>  | 47.53±1.27 <sup>Aa</sup> |
|                       | 5                  | 35.20±1.58 <sup>Ab</sup> | 37.89±0.96 <sup>Ab</sup> | 38.57±1.27 <sup>Bb</sup>  | 45.86±0.95 <sup>Aa</sup> |
|                       | 10                 | 26.68±2.22 <sup>Bb</sup> | 26.46±0.63 <sup>Bb</sup> | 27.13±0.95 <sup>Cb</sup>  | 33.18±0.63 <sup>Ba</sup> |
|                       | 15                 | 21.30±0.95 <sup>Cb</sup> | 26.46±1.27 <sup>Ba</sup> | 26.68±1.59 <sup>Ca</sup>  | 29.15±1.90 <sup>Ca</sup> |

<sup>1</sup>GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

<sup>2</sup>Means with different superscripts (<sup>A-C</sup> in the same column and <sup>a-d</sup> in the same row) differ significantly ( $p < 0.05$ ).

All values are means ± standard deviation for three replicates.

in the 1st day. The longer the storage duration, the lower the TPC ( $p>0.05$ ). This result was consistent with results of a previous study showing that TPC values of yogurt added with grape and callus extracts were decreased when storage period was longer (Karaaslan *et al.*, 2011). Temporary decrease of TPC in yogurt could be decomposition of polymeric phenolics in the presence of lactic acid bacteria during refrigerated storage (Dalling, 1986).

Reducing power values of all groups were decreased throughout the overall storage period compared to their initial values. This result was in agreement with results of Trigueros *et al.* (2014) showing that longer storage period decreased the reducing power of pomegranate yogurt. However, on day 15 of storage, reducing power values of GY0, GY1, GY3, and GY5 were not significantly different.

DPPH radical scavenging activity of GY5 and GY3 were at 47% and 44% each, which was the highest among all groups. After 15 d of refrigerated storage, DPPH radical scavenging activities of GY0, GY1, GY3, and GY5 groups were decreased to 21%, 26%, 27%, and 29%, respectively. This is consistent with results of a previous study showing that DPPH radical scavenging activity of yogurt added with grape and callus extracts yogurt after storage of 14 days is decreased 1.16-3.78 times (Karaaslan *et al.*, 2011).

It is generally known that antioxidant activities of plants are highly correlated with their phenolic compounds (Pérez-fons *et al.*, 2010), consistent with the results of this study. It has been reported that green olive has plenty of polyphenol compounds such as oleuropein (Amiot *et al.*,

1986). Hydrolysis of milk protein or organic acid production might have also contributed to the antioxidant activity of olive yogurt due to microbial metabolic activity during fermentation and refrigerated storage.

### Color measurements

Results of color L\*, a\*, and b\* values of yogurt after storage at 4°C for 15 d are summarized in Table 6. The higher the concentration of green olive added to yogurt and the longer the storage duration, the lower the value of L\*. When green olive was added at higher concentration, the value of a\* was decreased more. Because the color of green olive is dark green, if large amount of green olive is added to yogurt, values of L\* and a\* would be lower naturally. Furthermore, this is because green olive contains chlorophylls, pheophytins, and  $\beta$ -carotene that can decrease the value of a\* (Rahmani and Saari, 1991). Longer storage duration increased the value of a\* more. The more concentration of green olive powder increased, the more value of b\* increased ( $p<0.05$ ), and the longer period, the more value decreased generally ( $p>0.05$ ).

### Sensory evaluation

Sensory test of the manufactured yogurt was implemented through acceptability test after different amounts of green olive powder was added to yogurt. As shown in Fig. 1, GY3 yogurt color value was 4.63, which was the highest among all experimental groups. GY0 had the highest sour score. Sour scores of GY3 and GY5 groups were 3.88 and 3.25, respectively, without significant difference. Sour score of GY1 was the lowest. Texture scores

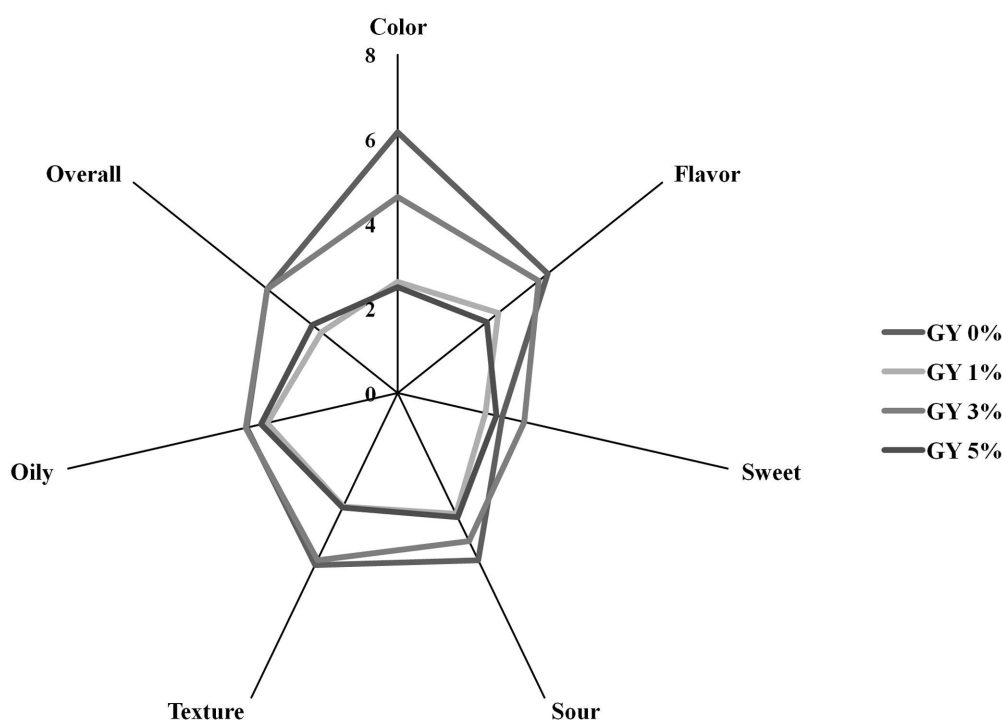
**Table 6. Color during storage of yogurt added with green olive**

| Color value | Storage period (d) | Treatment                |                          |                          |                          |
|-------------|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|             |                    | GY0                      | GY1                      | GY3                      | GY5                      |
| L*-value    | 1                  | 86.39±1.22 <sup>Aa</sup> | 80.58±1.44 <sup>Cb</sup> | 77.88±0.79 <sup>Dc</sup> | 72.86±2.47 <sup>Dd</sup> |
|             | 5                  | 85.91±1.19 <sup>Ba</sup> | 83.20±1.37 <sup>Ab</sup> | 78.39±1.01 <sup>Cc</sup> | 75.41±1.35 <sup>Cd</sup> |
|             | 10                 | 83.55±1.83 <sup>Ca</sup> | 81.80±1.65 <sup>Bb</sup> | 78.89±0.98 <sup>Bc</sup> | 78.89±1.78 <sup>Ac</sup> |
|             | 15                 | 83.77±1.01 <sup>Ca</sup> | 81.08±2.53 <sup>Cb</sup> | 79.32±1.22 <sup>Ac</sup> | 78.64±2.02 <sup>Bd</sup> |
| a*-value    | 1                  | -2.94±0.34 <sup>Ab</sup> | -2.69±0.37 <sup>Aa</sup> | -3.06±0.21 <sup>Cc</sup> | -3.45±0.43 <sup>Dd</sup> |
|             | 5                  | -3.36±0.13 <sup>Cd</sup> | -2.75±0.15 <sup>Bb</sup> | -2.62±0.27 <sup>Ba</sup> | -2.93±0.19 <sup>Cc</sup> |
|             | 10                 | -3.43±0.21 <sup>Dd</sup> | -2.84±0.20 <sup>Cc</sup> | -2.63±0.33 <sup>Bb</sup> | -2.2±0.26 <sup>Ba</sup>  |
|             | 15                 | -3.07±0.25 <sup>Bd</sup> | -2.68±0.17 <sup>Ac</sup> | -2.11±0.10 <sup>Ab</sup> | -1.92±0.36 <sup>Aa</sup> |
| b*-value    | 1                  | 7.40±0.61 <sup>Ac</sup>  | 16.79±0.08 <sup>Ab</sup> | 16.94±0.28 <sup>Ab</sup> | 24.33±0.16 <sup>Aa</sup> |
|             | 5                  | 7.13±0.12 <sup>Ad</sup>  | 10.21±0.10 <sup>Bc</sup> | 16.86±0.18 <sup>Ab</sup> | 20.47±0.24 <sup>Ba</sup> |
|             | 10                 | 6.33±0.17 <sup>Bd</sup>  | 9.60±0.30 <sup>Cc</sup>  | 14.37±0.18 <sup>Bb</sup> | 15.51±0.18 <sup>Ca</sup> |
|             | 15                 | 6.10±0.15 <sup>Bd</sup>  | 9.12±0.06 <sup>Cc</sup>  | 10.92±0.21 <sup>Cb</sup> | 14.36±0.18 <sup>Da</sup> |

<sup>1</sup>)GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

<sup>2</sup>)Means with different superscripts (<sup>A-D</sup> in the same column and <sup>a-d</sup> in the same row) differ significantly ( $p<0.05$ ).

All values are means ± standard deviation for three replicates.



**Fig. 1. Sensory test of yogurt added with green olive.** <sup>1</sup>GY0: control; GY1: yogurt added with 1% green olive; GY3: yogurt added with 3% green olive; GY5: yogurt added with 3% green olive.

of GY0 and GY3 were 4.5 and 4.38, respectively, without significant difference between the two. These scores were higher than those of the other two groups. Oily scores of all groups were not significantly different from each other. Flavor scores of GY0 and GY3 were 4.53 and 4.25, respectively, without significant difference between the two. Sweetness score of GY3 was 3.06 which was the highest among all groups. Overall scores of GY0 and GY3 were both 3.94, which were higher than those of GY1 and GY3 (at 2.31 and 2.59, respectively,  $p > 0.05$ ). Because sweetness can influence sensory characteristics, the overall value of GY3 is found to be the highest.

## Conclusions

Adding green olive powder to yogurt for intensifying fiber improved the antioxidant function. GY3 and GY5 were indicated higher viscosity than that in GY0 and GY1 until 5 d stored. Total count of lactic acid bacteria was no significant among experimental group except GY5 until 10 d. Antioxidant activity in green olive powder added yogurt was higher than that in GY0 during 15 d. Overall scores of GY0 and GY3 were both 3.94 in sensory evaluation. Yogurt added 3% of the green olive pow-

der has better antioxidant effect than GY0. In sensory evaluation, it shows similar score with GY0. This study showed that yogurt added 3% of green olive produced the acceptable product that influenced to substantial helpful health. Therefore, potential functionality of yogurt added green olive can be confirmed.

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