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# *Influence of purple basil extract (Ocimum basilicum L.) on chemical composition, rheology and antioxidant activity of set-type yoghurt*

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## Abstract

Four different types of yoghurt were manufactured using purple basil which was added to milk as water extract or powder forms at levels of 1.0 % and 0.4 % (w/w). Physicochemical and rheological properties, antioxidant capacity, color and total phenolic contents of yoghurt samples were measured during 21 days of storage. None of changes were observed for titratable acidity, pH and hardness parameters of yoghurt samples; however, some changes were observed during storage. The contents for total phenolic compounds and antioxidant activity [2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt ($ABTS^{+*}$) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH*) values] were dramatically higher in powder-added yoghurt samples than those of its water extract counterparts, while the lowest total phenolics and $ABTS^{+*}$ were observed in control sample during storage. The values for storage and loss modulus were higher in water extract-added samples than powder-added counterparts. In conclusion, no significant effect of the addition of powder or water extract of basil was observed on gross chemical characteristics of yoghurt, while use of basil powder positively contributed to antioxidant activity and changed the rheological behavior of yoghurt gel.

***Key words:*** **yoghurt, *Ocimum basilicum*, basil, antioxidant activity, rheology**

## Introduction

Fermented milk products already have a beneficial action due to its viable bacteria, calcium and other mineral matter and also vitamins (Heller, 2001). However, its functional effects are scarcely and plants or additives have been used to gain some functional or over-nutritional properties. It was reported that different food ingredients have been commonly added to increase its functionality and antioxidant capacity (Coïsson et al., 2005; Trigueros et al., 2011; Jiménez et al., 2008). Recently, many researchers interested in phenolic compounds due to their antioxidant, anti-inflammatory, anti-mutagenic and anti-clotting power which has been correlated with a declined risk of cardiovascular diseases and cancer development. Despite yoghurt having multi-nutritional characteristics and a great importance in human diet, it is not being considered as a major source of phenolic compounds. The amount of phenolics in dairy products is extremely restricted, which may be because of contamination of food production equipment with sanitizing agents and bacterial decomposition of proteins in milk. Thus, plant-based additives had been applied to improve the phenolic content of yoghurt (Gahruie et al., 2015). Anthocyanins belong to secondary plant metabolites within the class of polyphenols known as flavonoids. Anthocyanin containing ingredients are used in a variety of products such as beverages, jams, jellies, ice cream, yoghurt and confectionery (Scotter, 2015).

The *Ocimum* genus (Lamiaceae) consists of annual and perennial herbs and scrubs native to the tropical and subtropical regions of Asia, Africa and Central South America (Labra et al., 2004). *Ocimum basilicum* or Basil is called as "feslegen" or "reyhan" in Turkish (Chalchat and Ozcan, 2008; Giachino et al., 2014). Basil contains high levels of phenolic acids that contribute to its strong antioxidant capacity (Kwee and Niemeyer, 2011). Antioxidant is an increasingly important ingredient in food processing. Extracts of aromatic and medicinal plants are known to have some biological activities, particularly antibacterial, antifungal and antioxidant properties (Veronezi et al., 2014).

The polyphenols with plant origin have health benefit and also potential antioxidant source. In recent works and industrial applications indicated that use of antioxidant-rich plants products in yoghurt production improved quality characteristics and consumers' interests. It was hypothesized that the use of purple basil in the manufacture of set-type yoghurt with enhancing health benefit and aroma due to a strong volatile composition of purple basil. In this context, the main objectives of this study were to develop a new and functional yoghurt with containing natural flavor and polyphenols. The chemical composition, rheological properties, color, antioxidant activity and texture of purple basil-added yoghurt samples were compared during 21 days of storage to understand any contribution of water extracts or powder form of purple basil on the above characteristics of yoghurt.

## *Materials and methods*

### Basil samples

Basil, which is geographically indicated plant in 2017, was obtained from Arapgir (a county of Malatya, Turkey) and it was shade-dried after harvesting. Dried powder basil was used at levels of 0.4 % (for P04 sample) and 1.0 % (for P1 sample). Also, dried basil (1 g) was mixed with 9 mL pure water and it was rest for extraction at 70 °C overnight. After extracting, the mixture was centrifuged at 2000 rpm at 4 °C for 10 min and the supernatant was used in yoghurt production at levels of 0.4 % (v/v) and 1.0 % (v/v).

### Yoghurt manufacture

Two replicate trials were carried out in the manufacture of yoghurt. Cow's milk (Chemical composition and pH of raw milk were: 13.02±0.02 % for total solid, 3.67±0.±6 % for fat, 6.61±0.01 for pH, 2.75±0.07 % for protein and 0.85±0.01 % for ash) was heat-treated at 85 °C for 5 min and cooled to 47 °C. The milk was divided into five parts and water extract or powder of basil was added at levels of 0 % (free of basil) as control, 1.0 % (E1) and 0.4 % (E04) water extract, 1 % (P1) and 0.4 % (P04) powder forms. After blending the milk with water extract or powder, the mixtures were separately homogenized using an Ultra Turrax blender (IKA, model T25, Staufen, Germany) at 14,000 rpm until all ingredients were mixed completely. Samples were inoculated with yoghurt starter culture (YO-MIX, DANISCO FRANCE SAS 38360 Sassenage-FRANCE) at a ratio of 3 % (w/v), dispersed into plastic cups, *ca.* 200 g, and incubated at 43±1 °C until pH 4.7. Following incubation, all samples were kept at room temperature (21 °C) for 30 min and then moved to a cold room. The yoghurt samples were stored at 4 °C for 21 d and sampled after 1, 7, 14, or 21 days of storage.

### Physicochemical analysis in yoghurt samples

Total solid, protein, fat and ash contents and pH in milk used in production and yoghurt were determined by the methods described in Sahan at al. (2008).

Texture profile analysis (TPA) of the yoghurt samples was carried out after 1, 7, 14 or 21 days of storage using a Texture Analyzer Model LF Plus (Lloyd Instruments Ltd., Hampshire, UK). For texture analysis, the yoghurt samples were kept in plastic containers (45 mm diameter) of 80 mL (4±1 °C) and the analysis was carried out 2.0 mm $s^{-1}$ speed and

20 mm the distance covered in the sample using a cylindrical probe (10 mm diam. and 50 mm height). Four repetitions were realized for each sample. Water holding capacity (WHC) of the yoghurt samples was determined by the method given its detail in Sahan at al. (2008).

### Determination of antioxidant capacity by ABTS$^{+}$* method

The ABTS$^{+}$* [(2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)] was assayed by the method described in Re et al. (1999). The yoghurt samples were diluted with ethanol at a ratio of 1:9 (test sample), homogenized using Ultra Turrax blender (IKA, model T25, Staufen, Germany) and then centrifuged (Hettich, model 320R, Tutlingen, Germany) at 13500 rpm, 4°C for 10 min. It was mixed with 0.1 mL of ethanolic extract and 2.4 mL of ABTS$^{+}$* solution, and then read at 734 nm (Shimadzu, model uv-1800, Kyoto, Japan). The results were expressed as mmol Trolox equivalent antioxidant capacity (TEAC) on g of weight sample.

### Evaluation of DPPH* radical scavenging activity

DPPH$^{*}$ (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity was determined measured by the method as described in Chen et al. (2000) using the same spectrophotometer given section 2.4. The yoghurt samples were diluted with methanol at a level of 1:4 (test sample), homogenized and centrifuged at 13500 rpm (4 °C) for 10 min. A 0.1 mL of methanolic supernatant was mixed with 3.9 mL of DPPH$^{*}$ solution and the absorbance of the mixture were measured at 515 nm against methanol after 45 min in dark. The results were expressed as % DPPH$^{*}$ inhibition.

### Total phenolics, tartaric acid esters, flavonols and anthocyanin assays

Total phenolic contents of yoghurt samples were determined by the Foline-Ciocalteau procedure using gallic acid as standard with slight modifications (Demir et al., 2014). A 5 g yoghurt sample was mixed with 25 mL of methanol and centrifuged at 3000 ×g at 4 ºC for 25 min. A 1000 µL of supernatant was mixed with 3.16 mL of distilled water and also with 200 µL of Foline-Ciocalteau's reagent (Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA). The mixture was vortexed (Heidolph Instruments, D-91126 Schwabach, Germany) for 1 min and kept for 5 min at room temperature followed by addition of 600 µL of 2 % aqueous sodium carbonate. The absorbance was measured at 760 nm after 120 min incubation in dark at 20 °C against blank. The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) $g^{-1}$ dry weight.

Tartaric acid esters, flavonols and anthocyanins were determined by the methods described in Mazza et al. (1999). Standards curves were prepared using caffeic acid in 10 % ethanol (v/v) for tartaric esters, quercetin in 95 % ethanol (v/v) for flavonols and malvidin-3-glucoside in 10 % ethanol (v/v) for anthocyanins (Mazza et al., 1999)

### Color

Color of yoghurt samples was determined using a colorimeter (Chroma Meter CR-5, AC-A305, Konica Minolta, Osaka, Japan) with a 30 mm petri dish with illuminant D-65 and adjusted observer of 10° angle. The color which expressed as CIE $L^{*}$, $a^{*}$, $b^{*}$, *Chroma*, and hue angle (*h*°). All measurements were repeated three times.

### Rheological analysis

The rheological analyses of yoghurt samples were carried out using rheometer (Anton Paar, model MCR301, Anton Paar GmbH, Anton-Paar-Str. 20, Graz, Austria) during storage. The flow curves and the oscillatory assays was performed using a stainless steel cone and plate geometry with 25 mm of diameter and a gap of 1 mm, determining the flow curves and oscillatory trials. The yoghurts were loaded on the inset plate and the temperature was maintained at 5 °C±0.1 during the tests. The flow curves of the yoghurts were determined by scanning between deformation rates of 0 and 300 1 $s^{-1}$. The oscillatory trials were carried out with maximum deformation of 1 % and the frequency varied between 0.01 and 10 Hz. The parameters assessed in all samples were storage (G') and loss (G'') moduli.

### Statistical analysis

The data were analyzed using a package program SPSS v. 16.0 statistical software (SPSS Inc., Chicago, IL, USA) for windows. All analyses were performed at two trials and results were given as mean ± standard deviation. To compare the significant means, Duncan's multiple-range tests were used at P<0.05.

## Results and discussion

### Chemical composition

Total solid, protein, fat and ash contents of raw milk used in yoghurt production were: 13.08 %, 2.83 %, 3.65 % and 0.85 %, respectively. These values were regular constituents for cow's milk and in accordance with the regulations (Anonymous, 2000) for raw cow's milk in Turkey. Gross chemical composition and pH of yoghurt samples are shown in Tables 1 and 2. Total solid contents in yoghurt samples with water extract (E1 or E04) were almost the same; however, basil powder-added yoghurt samples (P04 and P1) contained higher levels of total solid than other samples (P<0.05). The values for total solid in yoghurt samples changed from 13.29-14.00 % and use of purple basil at powder form increased the total solid contents of yoghurt at significant level (P<0.05). The levels of protein in control and water extract-added (E1 or E04) yoghurt samples were almost the same; however, powder-added samples (P1 and P04; 3.26 % and 3.23 %, respectively) contained significantly higher levels of protein than those of control (3.07 %) or water extract-added (3.06 % and 3.02 % or E1 and E04 samples, respectively) yoghurt. Fat contents of these samples were found to be the same levels in the samples (3.00 % for control, E1 and P1 samples) with exception for P04 (2.80 %) and E04 (2.80 %) yoghurt samples. Also, no differences were observed for ash contents of the samples (P>0.05).

**TABLE 1.** Chemical composition (mean ± SD) of yoghurt samples with addition (1.0 or 0.4 %) of purple basil in water extract (E1 or E04) or powder (P1 or P04) form

| | Total solid content, % | Protein, % | Fat, % | Ash, % |
|---|---|---|---|---|
| Control | 13.29±0.03$^a$ | 3.07±0.00$^a$ | 3.00±0.00$^a$ | 1.02±0.11$^a$ |
| E1 | 13.49±0.14$^a$ | 3.06±0.13$^a$ | 3.00±0.00$^a$ | 1.09±0.05$^a$ |
| E04 | 13.31±0.07$^a$ | 3.02±0.16$^a$ | 2.80±0.00$^b$ | 1.05±0.01$^a$ |
| P1 | 14.00±0.16$^b$ | 3.26±0.01$^b$ | 3.00±0.00$^a$ | 1.11±0.03$^a$ |
| P04 | 13.75±0.16$^b$ | 3.23±0.03$^b$ | 2.80±0.00$^b$ | 1.02±0.06$^a$ |

$^{ab}$Values with different letter within the same column (samples) are significantly different (P<0.05)

**TABLE 2.** Physical and chemical characteristics (mean±SD) of samples with addition (1.0 or 0.4 %) of water extract (E1 or E04) or powder (P1 or P04) of purple basil during storage

| | Storage | Control | E1 | E04 | P1 | P04 |
|---|---|---|---|---|---|---|
| pH | 1 | 4.29±0.00$^{a,B}$ | 4.34±0.03$^{a,C}$ | 4.29±0.01$^{a,C}$ | 4.37±0.03$^{a,C}$ | 4.33±0.03$^{a,C}$ |
| | 7 | 4.18±0.00$^{a,AB}$ | 4.21±0.04$^{a,B}$ | 4.18±0.00$^{a,B}$ | 4.27±0.03$^{a,B}$ | 4.25±0.05$^{a,BC}$ |
| | 14 | 4.08±0.05$^{a,A}$ | 4.14±0.02$^{ab,AB}$ | 4.11±0.01$^{ab,AB}$ | 4.20±0.02$^{b,AB}$ | 4.14±0.00$^{ab,AB}$ |
| | 21 | 4.04±0.09$^{a,A}$ | 4.09±0.00$^{a,A}$ | 4.06±0.04$^{a,A}$ | 4.15±0.00$^{a,A}$ | 4.10±0.00$^{a,A}$ |
| Titratable Acidity[1] | 1 | 0.82±0.07$^{b,A}$ | 0.83±0.04$^{b,A}$ | 0.77±0.02$^{a,A}$ | 0.84±0.03$^{b,A}$ | 0.83±0.05$^{b,A}$ |
| | 7 | 0.95±0.00$^{a,B}$ | 1.01±0.00$^{a,B}$ | 0.99±0.02$^{a,B}$ | 0.96±0.08$^{a,AB}$ | 0.96±0.06$^{a,AB}$ |
| | 14 | 1.08±0.05$^{a,B}$ | 1.13±0.06$^{ab,B}$ | 1.08±0.10$^{a,B}$ | 1.24±0.05$^{b,B}$ | 1.19±0.05$^{b,B}$ |
| | 21 | 1.07±0.01$^{a,B}$ | 1.08±0.05$^{a,B}$ | 1.10±0.03$^{a,B}$ | 1.07±0.22$^{a,AB}$ | 1.02±0.27$^{a,AB}$ |
| °Brix | 1 | 7.70±0.28$^{ab,B}$ | 7.05±0.07$^{a,A}$ | 7.70±0.14$^{ab,B}$ | 8.15±0.07$^{b,B}$ | 7.40±0.14$^{ab,B}$ |
| | 7 | 7.30±0.14$^{a,B}$ | 7.30±0.14$^{a,B}$ | 7.35±0.21$^{a,B}$ | 7.60±0.28$^{b,B}$ | 7.30±0.14$^{a,B}$ |
| | 14 | 7.10±0.14$^{b,A}$ | 7.15±0.07$^{b,AB}$ | 7.05±0.07$^{a,A}$ | 7.00±0.00$^{a,A}$ | 7.05±0.07$^{a,A}$ |
| | 21 | 7.08±0.11$^{ab,A}$ | 7.10±0.14$^{ab,AB}$ | 7.00±0.00$^{a,A}$ | 7.20±0.00$^{b,A}$ | 6.95±0.21$^{a,A}$ |
| WHC (%) | 1 | 46.27±0.84$^{a,AB}$ | 51.00±0.00$^{c,C}$ | 49.00±0.00$^{b,C}$ | 46.00±0.00$^{a,D}$ | 47.50±0.71$^{ab,B}$ |
| | 7 | 48.37±0.23$^{d,B}$ | 45.93±0.63$^{b,B}$ | 47.86±0.41$^{cd,BC}$ | 40.96±0.63$^{a,C}$ | 46.15±0.33$^{bc,B}$ |
| | 14 | 44.42±0.42$^{b,AB}$ | 43.45±1.56$^{b,AB}$ | 43.37±1.67$^{b,AB}$ | 37.32±0.97$^{a,B}$ | 41.00±1.41$^{b,A}$ |
| | 21 | 39.57±0.10$^{b,A}$ | 39.78±1.10$^{b,A}$ | 37.38±0.87$^{b,A}$ | 33.33±0.09$^{a,A}$ | 39.40±0.27$^{b,A}$ |
| Hardness (N) | 1 | 0.15±0.02$^{a,A}$ | 0.17±0.01$^{a,A}$ | 0.16±0.02$^{a,A}$ | 0.13±0.04$^{a,A}$ | 0.17±0.05$^{a,A}$ |
| | 7 | 0.16±0.02$^{a,A}$ | 0.15±0.02$^{a,A}$ | 0.18±0.02$^{a,A}$ | 0.15±0.04$^{a,A}$ | 0.17±0.05$^{a,A}$ |
| | 14 | 0.20±0.06$^{a,A}$ | 0.19±0.01$^{a,A}$ | 0.21±0.00$^{a,A}$ | 0.16±0.05$^{a,A}$ | 0.18±0.06$^{a,A}$ |
| | 21 | 0.21±0.10$^{a,A}$ | 0.18±0.05$^{a,A}$ | 0.19±0.06$^{a,A}$ | 0.17±0.06$^{a,A}$ | 0.17±0.08$^{a,A}$ |

[1]Titratable acidity was expressed as g lactic acid 100 $g^{-1}$.
$^{a,b,c,d}$Values with different letter within the same line (samples) are significantly different (P<0.05).
$^{A,B,C}$Values with different capital within the same column (storage) are significantly different (P<0.05).

Table 2 shows the changes in pH, titratable acidity, °Brix, water holding capacity (WHC) and hardness values of yoghurt samples during 21 d of storage. pH values of yoghurt samples gradually decreased during storage and it ranged from 4.34 to 4.37 and 4.04 to 4.15 after 1 and 21 days of storage, respectively. Addition of basil at powder or its water extract form did not significantly change the pH of yoghurt, with exception of 14$^{th}$ day (P>0.05). It was reported that same levels of pH were found to be in yoghurt samples studied by Gauche et al. (2009) and Akalin et al. (2012), who reported that some fluctuations were observed for the pH values in the samples during storage.

No significant differences (P>0.05) were observed between titratable acidity, which is expressed as g of lactic acid in 100 $g^{-1}$, of yoghurt samples during storage. Titratable acidity, which was changed between 0.77 to 1.24 %, in yoghurt samples, increased during storage. Akalin et al. (2012) reported that no significant changes were observed in the titratable acidity after 1, 21 and 28 d of storage.

Brix values in the samples changed between 6.90-7.65 and the addition of powder basil changed the Brix values of yoghurt samples (P<0.05). However, these changes were observed when the basil added at a level of 1% at powder form after 1 and 7 days of storage.

Differences in WHC values of basil-added and control yoghurt samples were significant (Table 2) and the levels of the WHC of yoghurt samples gradually decreased during storage (P<0.05). The WHC values in powder-added yoghurt samples (P1 and P04) were lower than those of water extract-added yoghurt samples (E1 and E04), while control yoghurt had the lowest WHC values at the first day (P<0.05). Use of basil at powder form positively contributed to the serum separation in yoghurt at a substantial level (P<0.05). At the end of storage, the levels of WHC were almost the same for all samples and no differences were observed between the samples (P>0.05). These may probably be due to the stabilization of pH and texture (hardness) in yoghurt samples after 21 d of storage as shown Table 2. Lowering the pH (around 4.0 or 4.1) of milk during yoghurt fermentation likely resulted in a contraction of casein network. So, this contraction cause a greater whey separation due to the reduction of the net negative charge of casein micelles leading to a decrease in electrostatic repulsion between the charged molecules (Lee and Lucey; 2010). Hardness values of yoghurt samples changed between 0.13 to 0.21 N and these values did not change by storage time or treatments.

**TABLE 3.** Total phenolic contents (mean ± SD) of yoghurt samples with addition (1.0 or 0.4 %) of water extract (E1 or E04) or powder (P1 or P04) form of purple basil during storage (n=2)

| | Storage | Control | E1 | E04 | P1 | P04 |
|---|---|---|---|---|---|---|
| ABTS[1] | 1 | 0.67±0.01$^{a,D}$ | 1.76±0.01$^{d,C}$ | 1.17±0.01$^{b,B}$ | 2.94±0.04$^{e,B}$ | 1.42±0.02$^{c,C}$ |
| | 7 | 0.58±0.01$^{a,C}$ | 1.53±0.01$^{d,B}$ | 1.17±0.00$^{b,B}$ | 2.61±0.06$^{e,AB}$ | 1.31±0.01$^{c,B}$ |
| | 14 | 0.49±0.02$^{a,B}$ | 1.44±0.02$^{c,A}$ | 1.07±0.01$^{b,A}$ | 2.54±0.17$^{d,A}$ | 1.38±0.01$^{c,C}$ |
| | 21 | 0.42±0.01$^{a,A}$ | 1.46±0.02$^{c,A}$ | 0.96±0.01$^{b,A}$ | 2.42±0.03$^{d,A}$ | 1.19±0.02$^{c,A}$ |
| DPPH[2] | 1 | 10.66±0.26$^{a,C}$ | 41.91±0.09$^{d,B}$ | 33.16±0.17$^{c,B}$ | 43.42±0.17$^{d,C}$ | 25.32±0.17$^{b,C}$ |
| | 7 | 7.26±0.17$^{a,B}$ | 40.70±0.09$^{c,B}$ | 17.17±0.09$^{b,A}$ | 35.69±0.17$^{c,B}$ | 20.07±0.77$^{b,B}$ |
| | 14 | 7.04±0.77$^{a,B}$ | 37.69±0.09$^{d,AB}$ | 16.75±0.17$^{b,A}$ | 29.60±0.26$^{c,AB}$ | 17.96±0.17$^{b,AB}$ |
| | 21 | 6.19±0.26$^{a,A}$ | 31.71±0.34$^{d,A}$ | 15.36±0.09$^{b,A}$ | 25.98±0.09$^{c,A}$ | 15.67±0.17$^{b,A}$ |
| Total phenolic contents | 1 | 1.25±0.01$^{a,A}$ | 2.69±0.01$^{d,A}$ | 2.39±0.02$^{c,AB}$ | 3.10±0.01$^{e,A}$ | 2.08±0.02$^{b,A}$ |
| | 7 | 1.98±0.01$^{a,B}$ | 2.95±0.01$^{d,B}$ | 2.55±0.01$^{c,BC}$ | 3.42±0.02$^{e,B}$ | 2.08±0.02$^{b,A}$ |
| | 14 | 2.32±0.05$^{a,C}$ | 3.30±0.01$^{c,C}$ | 2.74±0.01$^{b,C}$ | 4.24±0.01$^{e,D}$ | 3.39±0.01$^{d,D}$ |
| | 21 | 2.00±0.01$^{a,B}$ | 3.36±0.01$^{c,D}$ | 2.25±0.02$^{b,A}$ | 3.88±0.01$^{d,C}$ | 2.64±0.01$^{b,B}$ |
| Tartaric acid esters[4] | 1 | 0.22±0.02$^{a,B}$ | 1.43±0.02$^{b,C}$ | 1.64±0.02$^{d,C}$ | 1.99±0.02$^{e,D}$ | 1.54±0.02$^{c,D}$ |
| | 7 | 0.14±0.02$^{a,A}$ | 1.20±0.02$^{d,B}$ | 0.64±0.02$^{b,B}$ | 1.74±0.00$^{e,C}$ | 0.97±0.00$^{c,C}$ |
| | 14 | 0.12±0.02$^{a,A}$ | 0.95±0.00$^{d,A}$ | 0.45±0.00$^{b,A}$ | 1.47±0.00$^{e,B}$ | 0.83±0.02$^{c,B}$ |
| | 21 | 0.09±0.02$^{a,A}$ | 0.89±0.00$^{c,A}$ | 0.45±0.00$^{b,A}$ | 1.11±0.00$^{d,A}$ | 0.74±0.00$^{c,A}$ |
| Total flavonols[5] | 1 | 0.09±0.00$^{a,B}$ | 0.24±0.00$^{c,B}$ | 0.17±0.00$^{b,B}$ | 0.36±0.00$^{d,B}$ | 0.32±0.00$^{d,C}$ |
| | 7 | 0.09±0.00$^{a,B}$ | 0.21±0.00$^{b,AB}$ | 0.14±0.00$^{ab,A}$ | 0.36±0.00$^{c,B}$ | 0.15±0.00$^{ab,B}$ |
| | 14 | 0.08±0.00$^{a,B}$ | 0.21±0.00$^{b,AB}$ | 0.14±0.00$^{ab,A}$ | 0.19±0.00$^{b,A}$ | 0.14±0.00$^{ab,B}$ |
| | 21 | 0.03±0.00$^{a,A}$ | 0.19±0.00$^{c,A}$ | 0.17±0.00$^{c,B}$ | 0.17±0.00$^{c,A}$ | 0.10±0.00$^{bc,A}$ |
| Total anthocyanins[6] | 1 | 0.03±0.00$^{a,A}$ | 0.37±0.00$^{c,B}$ | 0.22±0.00$^{b,B}$ | 0.68±0.00$^{d,D}$ | 0.22±0.00$^{b,B}$ |
| | 7 | 0.03±0.00$^{a,A}$ | 0.35±0.00$^{c,B}$ | 0.12±0.00$^{b,A}$ | 0.59±0.00$^{d,C}$ | 0.19±0.00$^{b,AB}$ |
| | 14 | 0.03±0.00$^{a,A}$ | 0.32±0.00$^{c,AB}$ | 0.09±0.00$^{a,A}$ | 0.37±0.00$^{d,B}$ | 0.15±0.00$^{b,B}$ |
| | 21 | 0.03±0.00$^{a,A}$ | 0.28±0.00$^{c,A}$ | 0.09±0.00$^{ab,A}$ | 0.28±0.00$^{c,A}$ | 0.12±0.00$^{b,A}$ |

$^{a,b,c,d,e}$Values with different letter within the same line (samples) are significantly different (P<0.05)
$^{A,B,C,D}$Values with different capital within the same column (storage) are significantly different (P<0.05)
[1]The values for ABTS (mmol as Trolox Equivalent (TE) kg$^{-1}$); [2]DPPH (percentage of inhibition); [3]total phenolics a or b (mg gallic acid equivalent (GAE) g$^{-1}$); [4]tartaric acid esters (mg caffeic acid g$^{-1}$); [5]total flavonols (mg quercetin acid 10 g$^{-1}$) and [6]total anthocyanins (mg malvidin-3-glucoside 10 g$^{-1}$) were expressed given in parentheses.

**TABLE 4.** Color measurements [*CIE L**, *a**, *b**, chroma, hue angle] (mean ± SD) of yoghurt samples with addition (1.0 or 0.4 %) of purple basil in extract (E1 or E04) or powder (P1 or P04) form during storage

| | Storage | Control | E1 | E04 | P1 | P04 |
|---|---|---|---|---|---|---|
| *L** | 1 | 94.82±0.40$^{e,A}$ | 76.57±2.42$^{c,A}$ | 85.01±0.01$^{d,A}$ | 58.18±0.17$^{a,A}$ | 72.93±0.53$^{b,A}$ |
| | 7 | 94.89±0.15$^{e,A}$ | 78.06±0.05$^{c,A}$ | 84.61±0.03$^{d,A}$ | 72.75±0.41$^{b,A}$ | 62.02±0.06$^{a,A}$ |
| | 14 | 95.23±0.15$^{e,A}$ | 73.81±0.46$^{c,A}$ | 85.10±0.10$^{d,A}$ | 59.75±0.27$^{a,A}$ | 71.39±0.52$^{b,A}$ |
| | 21 | 95.11±0.22$^{e,A}$ | 78.28±0.05$^{c,A}$ | 85.02±0.01$^{d,A}$ | 62.60±0.11$^{a,A}$ | 73.90±0.02$^{b,A}$ |
| *a** | 1 | -1.96±0.18$^{a,B}$ | 8.67±0.85$^{d,D}$ | 5.37±0.88$^{bc,B}$ | 6.42±0.18$^{c,A}$ | 3.95±0.01$^{b,A}$ |
| | 7 | -2.24±0.05$^{a,A}$ | 8.12±0.03$^{e,C}$ | 4.99±0.03$^{c,A}$ | 7.19±0.63$^{d,A}$ | 4.20±0.38$^{b,B}$ |
| | 14 | -2.24±0.03$^{a,A}$ | 7.97±0.28$^{c,A}$ | 4.91±0.05$^{b,A}$ | 7.54±0.23$^{c,A}$ | 4.23±0.19$^{b,B}$ |
| | 21 | -2.37±0.16$^{a,A}$ | 8.01±0.04$^{d,B}$ | 4.83±0.00$^{c,A}$ | 7.86±0.95$^{d,A}$ | 4.27±0.07$^{b,B}$ |
| *b** | 1 | 10.85±0.27$^{c,A}$ | 7.00±0.13$^{a,A}$ | 7.31±0.18$^{ab,A}$ | 6.88±0.05$^{b,AB}$ | 8.06±0.49$^{b,A}$ |
| | 7 | 11.13±0.13$^{e,A}$ | 7.08±0.04$^{a,A}$ | 7.76±0.04$^{b,A}$ | 7.15±0.74$^{b,B}$ | 9.34±0.79$^{c,B}$ |
| | 14 | 11.17±0.23$^{d,A}$ | 7.34±0.11$^{ab,B}$ | 7.96±0.02$^{b,B}$ | 6.91±0.06$^{c,AB}$ | 9.87±0.14$^{a,C}$ |
| | 21 | 10.73±0.21$^{d,A}$ | 7.47±0.01$^{a,B}$ | 8.07±0.02$^{ab,B}$ | 6.21±0.05$^{b,A}$ | 9.22±0.06$^{c,B}$ |
| Chroma (*C**) | 1 | 11.03±0.24$^{c,A}$ | 11.15±0.74$^{bc,B}$ | 9.10±0.38$^{a,A}$ | 9.41±0.09$^{ab,A}$ | 8.98±0.44$^{a,A}$ |
| | 7 | 11.35±0.12$^{e,A}$ | 10.78±0.05$^{d,A}$ | 9.23±0.02$^{a,AB}$ | 10.18±0.11$^{c,A}$ | 10.26±0.56$^{b,B}$ |
| | 14 | 11.39±0.22$^{c,A}$ | 10.83±0.13$^{b,A}$ | 9.35±0.01$^{a,B}$ | 10.23±0.14$^{ab,A}$ | 10.74±0.07$^{c,B}$ |
| | 21 | 10.99±0.19$^{c,A}$ | 10.95±0.03$^{c,A}$ | 9.40±0.01$^{a,B}$ | 10.69±0.05$^{bc,A}$ | 10.44±0.04$^{ab,B}$ |
| Hue angle (*h*°) | 1 | 100.27±1.18$^{c,A}$ | 40.98±0.01$^{a,A}$ | 58.31±0.01$^{ab,A}$ | 47.55±0.30$^{ab,B}$ | 63.04±0.00$^{b,A}$ |
| | 7 | 101.40±0.38$^{e,A}$ | 41.08±0.04$^{a,A}$ | 57.26±0.29$^{c,A}$ | 49.79±0.17$^{b,B}$ | 62.24±0.13$^{d,A}$ |
| | 14 | 101.34±0.36$^{e,A}$ | 42.67±1.43$^{b,AB}$ | 58.34±0.33$^{c,A}$ | 43.07±0.58$^{d,AB}$ | 67.44±0.48$^{a,A}$ |
| | 21 | 102.47±0.95$^{d,A}$ | 43.01±0.09$^{a,B}$ | 59.08±0.08$^{b,A}$ | 35.42±0.12$^{b,A}$ | 66.21±0.20$^{c,A}$ |

L*, a* and b* values implies in parenthesis (darkness/lightness), (-a green, +a red); (-b blue, +b yellow), respectively

$^{a,b,c,d,e}$Values with different letter within the same line (samples) are significantly different (P<0.05)

$^{A,B,C,D}$ Values with different capital within the same column (storage) are significantly different (P<0.05)

## Antioxidant activity and total phenolic contents

Table 3 shows the ABTS$^{+*}$ and DPPH$^{*}$ assays and total phenolic compounds of yoghurt samples. Use of basil in yoghurt production positively contributed to the antioxidant activity and phenolic compounds of the yoghurt samples (P<0.05). Use of water extract or powder of basil increased the ABTS$^{+*}$ values and the highest values for ABTS$^{+*}$ were observed in the powder-added (P1 or P04) and following water extract-added (E1 or E04) samples. Also, increased level of basil at water extract or powder form caused an increase in the ABTS$^{+*}$ at any storage time. ABTS$^{+*}$ value of yoghurt samples decreased during storage time, this may be due to a reducing of antioxidant power of the basil dispersed in yoghurt by the storage time. It was emphasized that the antioxidant activity of yoghurts may be enriched by incorporating some ingredients e.g., pomegranate peel extract, purple basil leaves, green tea, lemon, strawberry pulp (Jiménez et al., 2008; El-Said et al., 2014; Szymanowska et al., 2015).

Similar trends were observed for DPPH$^{*}$ values of the samples and its concentration dramatically increased by addition of basil with water extract or powder forms. DPPH$^{*}$ value of yoghurt samples declined over storage and this may be linked to the degradation of phenolic compounds with antioxidant activities and/or milk protein-polyphenol interaction (Amirdivani and Baba, 2011). DPPH$^{*}$

values of the yoghurt samples for E1 and P1 were higher levels than those of E04 and P04. The same parallel results were observed by $ABTS^{+*}$ assay with $DPPH^{*}$ as discussed above and a mid-strong correlation (~0.6 for correlation coefficient) was obtained. Chouchouli et al. (2013) reported that grape seed extract supplemented yoghurts exhibited a significantly higher radical scavenging capacity than that of control sample.

Possible use of phenolic compounds in dairy products such as yoghurt or ice cream has been proposed by O'Connell and Fox (2001) to enhance functionality of these dairy foods. Total phenolic contents (Table 3) in sample of E1 increased during 14 d of storage and then decreased after 21 d. The highest and lowest phenolic contents were observed in samples of P1 and control samples for all sampling times, respectively. Use of basil in yoghurt production caused an increase in phenolic content of yoghurt samples. The levels of gallic acid significantly higher in basil-added yoghurt samples and it decreased during storage time. As shown in Table 3, sample of P1 and control yoghurt had the highest and lowest gallic acid contents, respectively. Control yoghurt contained the lowest levels of flavonols and anthocyanin contents during storage, while P1 sample *vice versa*. Use of basil at a level of 1 % significantly increased the total tartaric acid esters, flavonols and anthocyanins. These compounds decreased for all samples towards the end of storage with exception of anthocyanin content in control sample. The anthocyanin content of control yoghurt did not change over storage. Chouchouli et al. (2013) reported that yoghurts fortified with grape seed extracts contained higher levels of polyphenols and exhibited higher antiradical and antioxidant activity than control sample. Also, it was stated that total phenolic content increased along with increasing levels of dried grape pomace in yoghurt samples (Mohamed et al., 2014).

## Color

Table 4 shows the color properties of yoghurt samples during 21 d of storage. Color in food is occasionally the first sensory and/or preference characteristic perceived by the consumers and tends to modify other perceptions such as taste and aroma (García-Pérez et al., 2005). Ayar and Gurlin (2014) pointed out that the color properties of yoghurt were significantly influenced by addition of some fruits and herbs. In this study, control yoghurt exhibited the highest $L^*$ value during storage, while the L* values decreased by addition of basil. The $L^*$ value which was lower in P1 and P04 yoghurt samples than E1 and E04 samples and also increasing level of basil caused a decreases of L* values (lightness) as shown in Table 4. It was thought that brightness decreased with the addition levels of basil; when it was powder form caused a sharp decrease in $L^*$ value. The $a^*$ value is an estimation of food foliage or redness and it was the highest in E1 yoghurt during storage. Use of basil and its level significantly influenced the $a^*$ value which decreased gradually in E04 yoghurt; however, it increased in P1 and P04 samples. Negative $a^*$ values were observed in control samples during storage, reflecting none of redness in control sample. However, it was expected that a certain level of redness may be observed in basil added samples and this was confirmed by this measurement (Table 4). It is noticeable that the storage did not influence the $a^*$ values. The $b^*$ value is an estimation of yellowness or blueness and the highest values were observed in control yoghurt during storage. Use of purple basil declined the $b^*$ values and increasing level of basil caused a lower level of $b^*$ which was higher in E04 and P04 yoghurts than the samples of E1 and P1. $C^*$ value is an estimation of food color saturation and $h°$ denotes hue angle, an angular measurement. Use of purple basil changed the values for $C^*$ and $h°$; i.e., these parameters were lower in basil added yoghurt than control sample ($P<0.05$). Increasing levels of basil caused a decrease in $h°$ values (Tab. 4). Similarly, use of grape extract in yoghurt production caused a darker and more reddish yoghurt than control sample (Chouchouli et al., 2013). Also, García-Pérez et al. (2005) reported that increasing level of orange fiber in yoghurt production caused an increase a* (less greenness) and b* values (more yellowness) and a decrease in L* values (less whiteness).

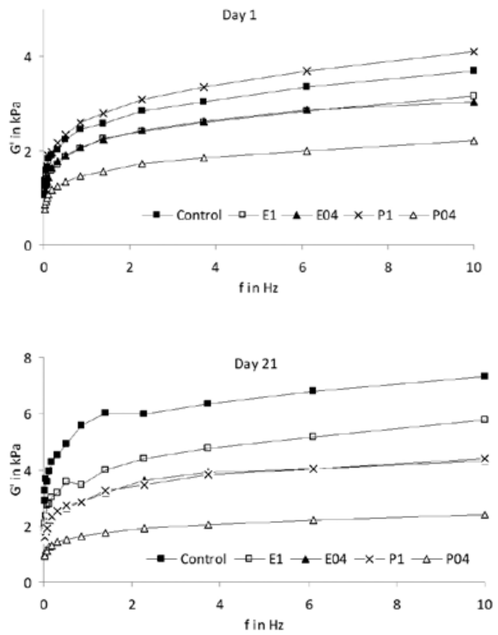


**FIGURE 1.** Storage (*G'*) of yoghurt samples prepared by addition (1.0 or 0.4 %) of purple basil in water extract (E1 or E04) or powder (P1 or P04) form after 1 and 21 days of storage

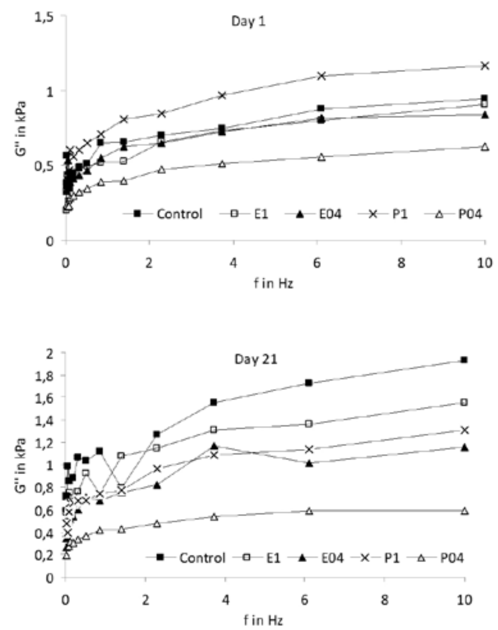


**FIGURE 2.** Loss (*G''*) modulus of yoghurt samples prepared by addition (1.0 or 0.4 %) of purple basil in water extract (E1 or E04) or powder (P1 or P04) form after 1 and 21 days of storage

### Dynamic rheological properties of yoghurt samples

Storage (*G'*) and loss (*G''*) moduli describe the elastic and viscous properties of a gelling system, respectively (Figs. 1 and Fig. 2). For the yoghurt samples, there is small frequency dependence in the linear-viscoelastic region. This means that the moduli (*G'* and *G''*) become non-linear at smaller strains as the frequency is increased. Beyond this linear region, the elastic structure begins partially to break down (Benezech and Maingonnat, 1994). *G'* value of yoghurt samples had higher than *G''* value. E1, E04 and P1 yoghurts exhibited maximum *G'* values at 14 d of storage, while P04 yoghurt was at maximum *G'* value after 7 d of storage. There were no significant differences in both *G'* and *G''* values for P1 yoghurt at any sampling time. Almost the same *G''* values were observed in 1 and 7 days-old E1 yoghurt samples, and the same trends were recorded after 14 and 21 d of storage. The highest and lowest *G'* and *G''* values were measured for P1 and P04 yoghurt samples at the first d of storage, respectively. Both *G'* and *G''* values of yoghurt samples varied depending on storage time ($P<0.05$). Values for *G'* and *G''* in the samples (P1 and P04) was lower than yoghurts (E1 and E04) over storage time. Guggisberg et al. (2009) studied rheological behavior of low-fat and whole milk set-type yoghurt with addition of inulin. These authors pointed out that the addition of inulin and the level of fat in milk significantly affected the rheological behavior of yoghurt samples. Ramirez-Santiago et al. (2010) studied the effect of soluble dietary fiber from *Pachyrhizus erosus* L. on rheological properties of stirred-type yoghurt and these authors reported that yoghurt with yam soluble fiber extracted from *Pachyrhizus erosus* L. resulted in lower storage (*G'*) and loss modulus (*G''*) in the linear viscoelastic region than the stirred yoghurt. The effect germinated soybeans on the rheological characteristics of soy yoghurts was studied and it was reported that yoghurt with soybean germination resulted in lower storage (*G'*) and loss (*G''*) modulus than yoghurt from ungerminated soybean (Yang et al., 2012).

## Conclusions

Use of basil at water extract or powder form did not significantly change the physicochemical properties of yoghurt, while it (especially powder form) positively contributed the antioxidant activity, total phenolic content, levels of tartaric acid esters, flavonols and anthocyanin of yoghurt samples. Use of basil, at powder, in yoghurt production resulted in higher levels of $ABTS^{+*}$ and DPPH* than the water extract form. This may be linked to release of some compounds in basil into the yoghurt during extraction or fermentation of milk and its storage. So, the antioxidative substances dissolved well and remained in acidic pH of the final product. The use of basil at powder or water extract in yoghurt production also showed a significant effect on the color properties of yoghurt. Use of basil lowered the values for $L^*$, $b^*$, $C^*$ and $h°$ values, but increased $a^*$ value. $G'$ value of yoghurt samples had higher than $G''$ value. Both storage ($G'$) and loss ($G''$) modulus in control yoghurt increased during storage.

# *Utjecaj dodatka ekstrakta ljubičastog bosiljka (Ocimum basilicum L.) na kemijski sastav, reološka svojstva i antioksidacijski kapacitet čvrstog jogurta*


## Sažetak

U ovom istraživanju proizvedene su 4 vrste jogurta obogaćenog ljubičastim bosiljkom koji je dodavan u obliku vodenog ekstrakta ili kao prah u količinama 1,0 % i 0,4 % (w/w). Tako dobiveni uzorci jogurta skladišteni su 21 dan unutar kojih su im određivana fizikalno-kemijska i reološka svojstva, antioksidacijski kapacitet, boja i koncentracija ukupnih fenola. Nisu utvrđene promjene u titracijskoj kiselosti, pH vrijednosti i čvrstoći gruša, međutim, određene promjene zabilježene su prilikom skladištenja. Koncentracija ukupnih fenola i antioksidacijski kapacitet [diamonijeva sol 2,2'-azino-bis(3-etilbenzotazolin-6-sulfonske kiseline ($ABTS^{+*}$) i 2,2-difenil-1-pikrilhidrazil($DPPH^*$)] bili su znatno veći u uzorcima jogurta kojima je dodavan prah u usporedbi s uzorcima kojima je dodavan vodeni ekstrakt, dok je najniža koncentracija ukupnih fenola i najniža ABTS vrijednost utvrđena u kontrolnom uzorku prilikom skladištenja. Vrijednosti određivanih reoloških parametara za promjene tijekom skladištenja i gubitak konzistencije bili su veći kod uzoraka s dodatkom vodenog ekstrakta. Zaključno, nije utvrđen značajan utjecaj dodatka praha ili vodenog ekstrakta bosiljka na kemijski sastav i svojstva jogurta, dok je pozitivno utjecao na antioksidacijski kapacitet i izmijenio reološka svojstva jogurta.



***Ključne riječi:*** **jogurt, *Ocimum basilicum,* bosiljak, antioksidacijski kapacitet, reologija**


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