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Fortification of set-type yoghurts with *Elaeagnus angustifolia* L. flours: Effects on physicochemical, textural, and microstructural characteristics

Hale İnci Öztürk[a,*], Sümeyye Aydın[a], Didem Sözeri[b], Talha Demirci[a], Durmuş Sert[c], Nihat Akın[a]

[a] Department of Food Engineering, University of Selcuk, Konya 42050, Turkey
[b] Department of Food Engineering, University of Namık Kemal, Tekirdag 50860, Turkey
[c] Department of Food Engineering, University of Necmettin Erbakan, Konya 42060, Turkey

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ABSTRACT

The effect of peeled oleaster (*Elaeagnus angustifolia* L.) flour (PO) and unpeeled oleaster flour (UPO) in different levels (1% and 2%) on the quality parameters of set type yoghurt was investigated throughout 28 days of storage. Acidification kinetics, water holding capacity, syneresis level, textural characteristics and microstructure were firstly measured. Additionally, influence of oleaster flours on DPPH and $ABTS^+$ radical scavenging activity and total phenolic content of yoghurt samples was also assessed. Besides, all the products were subjected to sensorial preference test. Enrichment with 2% PO and UPO reduced fermentation time by 31 and 37 min, respectively. Addition of PO and UPO increased cohesiveness and the viscosity index, furthermore, syneresis decreased. Oleaster supplementation to yoghurt significantly increased scavenging activities of DPPH and $ABTS^+$ radicals and the highest activities were determined in yoghurts fortified with 2% UPO. The results demonstrated that yoghurt with reduced syneresis, improved functional properties, and enhanced some textural characteristics can be achieved by a 2% UPO addition. Also, surprisingly yoghurts containing 2% UPO had similar consumer overall preference scores comparing to plain yoghurt.

1. Introduction

Yoghurt is a fermented dairy product made by the activity of *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*L. bulgaricus*), and it is widely consumed around the world (Akın, 2006). Textural properties of yoghurt are very important for consumer acceptability, product development, and quality assessment (Benezech & Maingonnat, 1994). Yoghurt gel formation is caused by the aggregation of casein micelles after decreased pH in the milk due to lactic acid production. The three-dimensional protein network is directed by the total solids content, acidity production rate, and proteolytic activity of the starter bacteria (Lee & Lucey, 2004). Yoghurt gel structure is principally associated with the shape and strength of the protein network structure. Increasing the interconnections between milk proteins by reducing syneresis is an important approach in the development of the textural quality of any yoghurt (Shi, Han, & Zhao, 2016).

Some studies have been performed to enhance yoghurt quality such as reducing syneresis, enhancing viscosity and textural characteristics by adding whey proteins (Laiho, Williams, Poelman, Appelqvist, & Logan, 2017), fiber-rich fruit peel powder (do Espírito Santo, Perego, Converti, & Oliveira, 2012), seed mucilage or guar gum (Mudgil, Barak, & Khatkar, 2016), exopolysaccharides (Zhang, Folkenberg, Qvist, & Ipsen, 2015), and hydrocolloids such as gelatin (Shi et al., 2016).

Elaeagnus angustifolia L. which is called oleaster or Russian olive pertains to the genus *Elaeagnus* of Elaeagnaceae family. *E. angustifolia* L. has small reddish-brown elliptic fruits and grows in a wide geographical area including Asia, Europe and North America (Fonia, White, & White, 2009). Oleaster fruits have high nutritional value due to the comprising of proteins, carbohydrates, vitamins, minerals, phenolic compounds, antioxidants, and fibers (Abizov, Tolkachev, Mal'tsev, & Abizova, 2008). Oleaster fruit consists of 27.1% glucose, 22.3% fructose, 12% protein, 4.65% ascorbic acid and 1% ash (Akbolat, Ertekin, Menges, Guzel, & Ekinci, 2008; Ayaz & Bertoft, 2001). Also, phytochemical researches have shown that oleaster fruit is rich in flavonoids, phenolic acids and vitamins (Abizov et al., 2008; Ayaz & Bertoft, 2001), thus, oleaster fruits showed high antioxidant activity (Faramarz, Dehghan, & Jahanban-Esfahlan, 2015). Çakmakçı et al. (2015) determined an increase in total phenolic content and antioxidant activity of ice cream fortified with oleaster flour. Besides, Sahan et al. (2015) noted that oleaster flour with high fiber content (20–30%) showed high water absorption potential and an enhancing effect on emulsion stability.

* Corresponding author. Department of Food Engineering, Faculty of Agriculture, University of Selcuk, Konya 42050, Turkey.
E-mail address: haleinciozturk@selcuk.edu.tr (H.İ. Öztürk).

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Although there were some previous studies regarding many new ingredients to develop yoghurt quality parameters and add beneficial properties, we met no studies about the incorporation of oleaster as an ingredient in the yoghurt formulation. On the basis of this background, the present research was undertaken to examine the influence of oleaster flour supplementation on the textural, microstructural, physicochemical and sensory attributes of yoghurt. Moreover, the effects of oleaster fortification on acidification kinetics, total phenolic contents and antioxidant activity were evaluated.

2. Materials and methods

2.1. Experimental materials

Raw cow milk was collected from Selcuk University Dairy Farm, Konya, Turkey and medium-heat skim milk powder (34.5% protein, 3.5% moisture, 7.2% ash, 55% lactose, pH:6.55 and 0.112% titratable acidity) was provided from ENKA Dairy Product, Konya, Turkey. Commercial freeze-dried yoghurt starter culture YF-L901 consisting of *S. thermophilus* and *L. bulgaricus* supplied from Chr's Hansen-Peyma (Istanbul) was used in yoghurt production.

Two different supplements, including PO and UPO, were prepared. In the production of the PO, peeled and seedless oleaster fruits were kept in hot air oven at 30 °C over the night and then were ground in a hammer mill (Falling Number-3100 Laboratory Mill, Huddinge, Sweden). The same operations except peeling process were also carried out to obtain UPO.

2.2. Set-type yoghurt processing and sample treatments

Dry matter of milk was adjusted to 16% by adding medium-heat skim milk powder and it was then divided into 5 experimental groups. PO and UPO were added separately at 1% and 2% ratios to experimental groups except control sample. Each experimental mix was pasteurized at 90 °C for 10 min and then rapidly cooled to 42 °C. The yoghurt mixes were inoculated with 2% starter culture and they were then put into 150 mL sterile plastic containers. All containers incubated at 42 °C until the pH reached 4.5 and were stored at 4 °C for 28 days. Yoghurt samples were identified as follows: with peeled oleaster flour (YPO) and with unpeeled oleaster flour (YUPO).

2.3. Monitoring of acidification kinetics

The changes in pH values during fermentation were monitored until the pH 4.5. Four parameters were utilized to characterize the process kinetics: the maximum acidification rate (V_{max}, 10^{-3} upH min^{-1}), the time at which the maximum acidification rate (T_{Vmax}, h), the time required to reach pH 5.0 ($T_{pH\ 5.0}$, h), and the time required to complete fermentation ($T_{pH\ 4.5}$, h) (Marafon, Sumi, Alcantara, Tamime, & De Oliveira, 2011).

2.4. Assessment of physicochemical characteristics

Total solids, ash, fat and color characteristics of yoghurt samples were determined according to the methods described by Bradley et al. (1992) on day 14. Furthermore, titratable acidity, pH (Bradley et al., 1992), water holding capacity and syneresis (Isanga & Zhang, 2009) were observed at 1, 14, and 28 days of cold storage.

2.5. Extraction for antioxidant and total phenolic content analyses

For extraction, 5 g of yoghurt samples were mixed with 25 mL of 75% methanol solution. Thereafter the mixes were homogenized by ultra-turrax homogenizer (IKA T-25, Germany) and subjected to centrifugation (Nüve NF 800R, Turkey) at 7200 rpm for 10 min at 4 °C. Finally, the acquired supernatants were filtered by Whatman No.1 and the extracts were stored at 4 °C for analysis.

2.6. Assessment of antioxidant activity with radical scavenging methods

Antioxidant activities of yoghurt samples were determined by ABTS (2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic) (Re et al., 1999) and DPPH (1,1-diphenyl-2-picrylhydrazyl) (Brand-Williams, Cuvelier, & Berset, 1995) radical scavenging methods. ABTS radical scavenging activity was detected at 734 nm and the measured absorbances were converted to Trolox equivalent antioxidant capacity (TEAC). The results of ABTS radical scavenging activity were expressed as μM Trolox equivalent per gram of yoghurt samples. DPPH radical scavenging activity was examined at 517 nm and the results were given as % inhibition.

2.7. Measurement of total phenolic content

The total phenolic content (TPC) was determined by using the method of Tseng and Zhao (2013). Reaction absorbance was measured at 765 nm. The results, that were calculated based on the gallic acid curve, were expressed as microgram gallic acid equivalent (GAE) per gram of yoghurt samples.

2.8. Texture profile analysis and viscosity index

Firmness, consistency, cohesiveness and viscosity index values were obtained by TA-XT2 Texture Analyzer (Stable Micro Systems, England) equipped with a 500 N compression load cell and operating at 1 mm s^{-1} head speed. The probe was a 25-mm acrylic cylinder moved speed of 5 mm s^{-1} and test speed of 1 mm s^{-1} through 10 mm within the sample. Textural characteristics were examined over cold storage and the results were given as the mean of three measurements.

2.9. Microstructure analysis

After two weeks of storage, the confocal laser scanning microscopy (CLSM) analysis was carried out using an inverted confocal laser scanning microscope (A1/A1R, Nikon, Japan) equipped with Nikon Plan Fluor, PA:0.30 objective lens. For CLSM observation, yoghurt samples were stained with Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) in water solution (0.2%, w/v) for 30 min at room temperature. CLSM observation was carried out in a dark and an Ar laser line (488 nm) was employed as a light source to excite fluorescent dyes Rhodamine B. For the yoghurt analysis, at least two CLSM images were taken for each yoghurt treatment in each trial. Two trials of yoghurt were carried out and hence a minimum of four images were collected for each treatment.

2.10. Sensory evaluation

Sensory acceptability test of yoghurt samples was assessed by a trained panel of seven members using five-point hedonic scale (1: unacceptable; 5: excellent) (Karagul-Yuceer & Drake, 2006). Panelists appraised flavor, color and appearance, body and texture, odour, and general acceptability of yoghurt samples on the 14th day of storage.

2.11. Statistical analysis

The results were analyzed with one-way ANOVA to identify significant differences between means of samples and storage days. All data were expressed as a mean ± standard deviation of the three replicates. The means of results were compared by the Tukey test with a confidence interval set at 95%.