



## EMULSIONS OF ORANGE AND COCONUT OILS AND THEIR USE IN PEANUT BUTTERS

**Emin Burcin Özvural\***, Ebru Celen

Cankiri Karatekin University, Faculty of Engineering, Department of Food Engineering, Cankiri, Turkey

Received / Geliş: 10.12.2019; Accepted / Kabul: 21.05.2020 Published online / Online baskı: 30.05.2020

Özvural, E.B., Celen, E. (2020). Emulsions of orange and coconut oils and their use in peanut butters. GIDA (2020) 45(3) 544-554 doi: 10.15237/gida.GD19161

Özvural, E.B., Çelen, E. (2020). Portakal ve hindistan cevizi yağı emülsiyonları ve fıstık ezmelerinde kullanımları. GIDA (2020) 45(3) 544-554 doi: 10.15237/gida.GD19161

### ABSTRACT

In this study, orange essential oil and coconut oil emulsions and their mixtures were prepared and added to peanut butter to prevent lipid oxidation. Sodium caseinate was used as an emulsifier and ultrasonication was used for homogenization. Emulsions containing orange essential oil had a higher total phenolic content and antioxidant capacity than the coconut oil emulsions ( $P<0.05$ ). TBARS values of the peanut butters containing ultrasonicated emulsions were considerably higher than the other treatments ( $P<0.05$ ) at the end of storage (20<sup>th</sup> day). Samples containing ultrasonicated emulsions had lower L\* (lightness) values than the other samples ( $P<0.05$ ). Results showed that addition of these emulsions into peanut butters was effective in suppressing the lipid oxidation, but still further research is needed to produce the emulsions in nanoscale so as to increase the antioxidant properties of the oils.

**Keywords:** Peanut butter; orange oil; coconut oil, ultrasonication, emulsion

## PORTAKAL VE HİNDİSTAN CEVİZİ YAĞI EMÜLSİYONLARI VE FISTIK EZMELERİNDE KULLANIMLARI

### ÖZ

Bu çalışmada, portakal esansiyel yağı ve hindistan cevizi yağı emülsiyonları ve bunların karışımları hazırlanmış ve lipid oksidasyonunu önlemek amacıyla fıstık ezmelerine eklenmiştir. Emülgatör olarak sodyum kazeinat ve homojenizasyon tekniği olarak ultrasonikasyon kullanılmıştır. Portakal esansiyel yağı içeren emülsiyonların hindistan cevizi yağı içeren diğer emülsiyonlardan daha yüksek toplam fenolik içeriğine ve antioksidan kapasiteye sahip olduğu belirlenmiştir ( $P<0.05$ ). Depolamanın sonunda (20. gün) ultrasonikasyon uygulanmış emülsiyonları içeren örneklerin TBARS değerlerinin diğer denemelere göre oldukça yüksek olduğu gözlemlenmiştir ( $P<0.05$ ). Ultrasonikasyon uygulanmış emülsiyonları içeren örneklerin L\* (parlaklık) değeri diğer örneklerden daha düşük olarak belirlenmiştir ( $P<0.05$ ). Sonuçlar, bu emülsiyonların fıstık ezmesine ilave edilmesinin lipid oksidasyonunu baskılamada etkili olduğunu, ancak yağların antioksidan özelliklerini artırmak amacıyla emülsiyonların nano boyutta üretilmesine yönelik daha fazla araştırmaya ihtiyaç olduğunu göstermiştir.

**Anahtar kelimeler:** Fıstık ezmesi; portakal yağı; hindistan cevizi yağı, ultrasonikasyon, emülsiyon

\* Corresponding author / Yazışmalardan sorumlu yazar;

✉ bozvural@karatekin.edu.tr

☎ (+90) 376 218 95 00 /8355

☎ (+90) 376 218 95 36

Emin Burçin Özvural; ORCID no: 0000-0001-7919-2567

Ebru Çelen; ORCID no: 0000-0003-1286-5362

## INTRODUCTION

Emulsions are systems in which at least two immiscible liquids are dispersed in droplets. These systems have two phases, hydrophilic and lipophilic. The outer phase, called the continuous phase, carries the inner phase as droplets (İlyasoğlu and El, 2010). Nanoemulsions are emulsion classes with droplet sizes ranging from 20 to 100 nm; however, in some resources emulsions with particle sizes of 200 or 500 nm are also be considered as nanoemulsions (Donsi et. al., 2012; Abbaszadeh et. al., 2014). Nanoemulsions appear transparent and are more stable than regular emulsions to emulsion deterioration such as creaming, coalescence, flocculation and Ostwald droplet growth. Nanoemulsions are of interest in practical applications due to their physicochemical properties, small droplet sizes and long term stability (Lu et. al., 2018). Nanoemulsion systems are used in the food, pharmaceutical and cosmetic industries for the encapsulation of flavouring agents, bioactive components, and colorants (McClements, 2011). Application of nanotechnology techniques to foods can change the texture, taste, sensory properties, colour, processing ability and stability of foods during storage (McClements et. al., 2009; Huang et. al., 2010; Özogul et. al., 2016).

High energy and low energy homogenization methods are used in the preparation of nanoemulsions. Homogenizers, microfluidizers and ultrasonic devices are used in high energy methods, but spontaneous emulsification, phase inversion temperature, phase inversion composition and emulsion inversion point are known as low energy methods (Anton and Vandamme, 2009; Piorkowski and McClements, 2013; Özogul et. al., 2016). Ultrasonication is one of the high energy techniques used to prepare nanoemulsions and nanodispersions by forming nano-sized droplets and particles (Tan et. al., 2016; Ghasemi et. al., 2017). The passage of ultrasound through the liquid medium creates cavitation conditions that release large amounts of energy locally (Gogate et. al., 2006). Ultrasonic emulsification is a technique used to produce stable emulsion with low energy input (Canselier

et. al., 2002; Sivakumar et. al., 2014). Especially in terms of droplet size and distribution, it has been observed that the emergence of cavitation conditions plays an important role in determining the effectiveness of ultrasound-induced emulsification (Cucheval and Chow., 2008; Ramisetty et. al., 2015).

The accumulation of waste material (husk, kernel and pulp) is largely generated from the consumption of oranges commercially or at home and from the orange juice industry. The peel represents 50-65% of the fruit and causes a great environmental pollution. It is possible to extract oil from the peels as a material used in the aroma and flavouring industry (Martins et. al., 2013). Orange peel oil contains limonene (94%), myrcene (2%), linalool (0.5%), octanal (0.4%), decanal (0.4%), neral (0.1%), geraniol (0.1%) etc. (Hashtjin and Abbasi, 2015). Limonene is one of the most abundant terpenes in nature and is the major constituent of many essential oils from citrus. Limonene has a pleasant lemon-like odor and is therefore used as a flavouring and fragrance agent in fruit juices, candies, gums, soft drinks and ice creams. Phenolic compounds such as limonene show antioxidant effects by scavenging free radicals (Vieira et. al., 2018).

Coconut oil is a valuable oil with vitamin E, polyphenols and a wide variety of medium chain triglycerides such as lauric acid, myristic acid and caprylic acid (Jayadas and Nair, 2006). It shows many biological activities such as antioxidant, antitumor, antithrombotic and hypolipidemic effects. Considering all these benefits of coconut, it may be possible for people to make more use of this oil in nanoemulsions (Pengon et. al., 2018).

Peanut butter is defined as a paste-like dispersion (Co and Marangoni, 2012) or a highly concentrated suspension in which solid round peanut pieces are dispersed continuously in the oil phase (Carraeu et. al., 2002). The total lipid content of peanut butter is 47-50%, and peanut oil contains about 52% oleic acid, 32% linoleic acid, and the remainder contains saturated fatty acids (Tanti et. al., 2016). Salt, sweeteners, emulsifiers or stabilizers can be incorporated in

the production of peanut butter. Similar products added to the structure other than the standard product should be labeled (Sander et. al., 2014).

In this study, emulsions were formed by ultrasonication using orange essential oil, coconut oil and sodium caseinate. The total phenolic content and antioxidant properties of the emulsions were determined and then the emulsions were added into the peanut butters in different ratios. Both oils were separately added at the same proportions into the peanut butters without emulsification in order to make a comparison among the treatments. Oils and emulsion solutions added peanut butters were stored at room temperature for 20 days and quality characteristics of the products were monitored.

## **MATERIALS AND METHODS**

### **Materials**

The orange essential oil used in the study was obtained from the Ecem Naturel Cosmetics Personal Care and Health Products Company, Istanbul (Turkey) and pure coconut oil (Life In, Origo Gıda, Gaziantep, Turkey) was obtained from a local market. Peanuts (unsalted, shelled and slightly roasted) were purchased from a local nut shop in Cankiri, Turkey.

### **Preparation of Emulsions**

The oil phase of the emulsions was formed by mixing orange oil (essential oil) and coconut oil in different proportions (100: 0, 50:50, 0: 100, orange oil: coconut oil, v/v). The emulsions were prepared by mixing the oil phase and sodium caseinate (Sigma-Aldrich- C8654) solution (3%, w/w) at a ratio of 1: 3 (w/w), respectively. The coarse emulsions were prepared by Ultra-Turrax homogenizer at 11000 rpm for 5 minutes. After that, ultrasonication was applied to reduce the particle size of the emulsions. To this end, an ultrasonicator (Sonics VCX500, Newtown, CT, USA) with a probe (diameter: 13 mm) and a maximum power output of 20 kHz 500 W for a period of 10 minutes at 40% amplitude were used. The container containing the sample was kept in an ice bath during the process in order to prevent heating.

### **Particle size of emulsions**

The particle sizes of the prepared emulsions were measured by dynamic light scattering (DLS) method using Zeta sizer (Malvern Instruments, Malvern, UK). Three repetitions were conducted.

### **Total phenolic content**

The total phenolic content of the samples was colourimetrically measured using the Folin – Ciocalteu reagent by the modification of the method in Anton et. al., (2009). Accordingly, 0.5 g of the sample was mixed with 12.5 ml of acetone-water mixture (4: 1) (v / v) on a magnetic stirrer for 2 hours. The samples were then centrifuged at 3000xg for 12 minutes. The resulting supernatant was used for analysis. In the presence of phenolic compounds, colour change occurs by the reduction of Folin-Ciocalteu reagent with the aid of sodium carbonate. The supernatant obtained was diluted 1/10 and 0.2 ml of this sample was mixed with 1.5 ml of 10 times diluted Folin-Ciocalteu reagent. After 5 minutes, 1.5 ml of Na<sub>2</sub>CO<sub>3</sub> (60 g/l) was added to the mixture and the mixture was kept at room temperature in the dark for 90 minutes. Absorbance values were read at 725 nm using a UV-Visible spectrophotometer and the results were given in mg gallic acid equivalent (GAE)/g dry matter using the prepared gallic acid (3,4,5-Trihydroxybenzoic acid) standard curve. Three repetitions were performed for each sample.

### **DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging analysis**

DPPH radical solution was prepared by dissolving 5 mg of DPPH in 200 mL of 80% methanol. 500 µL of sample (obtained by extraction as described in total phenolic analysis) was added into 3.5 mL of the prepared methanol-DPPH solution and vortexed. After holding the mixture for an hour at room temperature and in the dark, absorbance of the mixture was measured at 517 nm on the spectrophotometer and the results were calculated using the following equation. The antiradical activities of the samples was given as % inhibition shown below (Brand-Williams et. al., 1995; Rodriguez et. al., 2002).

Mean value of three measurements was given for each treatment.

$$\% \text{ Inhibition} = (C - S/C) \times 100$$

C: Absorbance of the control at 517 nm

S: Absorbance of the sample at 517 nm

### ABTS analysis

The ABTS $\cdot$ + radical was formed by reacting 5ml of 7mM ABTS solution with 88 $\mu$ l of 140 mM potassium persulfate aqueous solution. The mixture was allowed to stand at room temperature in the dark for 16 hours before use. 1 ml of ABTS $\cdot$ + solution was diluted with ethyl alcohol to give an absorbance value of  $0.70 \pm 0.02$  at 734 nm. 700 $\mu$ l (10 mg / ml) of sample found in isopropyl alcohol was then added onto 3 ml of ABTS + radical and homogenized for 6 minutes. The absorbance reading was carried out at 734 nm

using ethyl alcohol as the blank sample. Antioxidant capacity is given as % inhibition, similar to DPPH analysis (Santos et. al., 2017).

### Preparation of peanut butter

Roasted and shelled peanuts (400 g) were ground in a food processor (Krupps Rotary 500, Germany) until the desired spreadability was obtained. After the pre-test, the time required for the formation of the desired creamy structure was determined as 5 minutes and the same time was applied to all samples. After the desired structure was formed, orange oil, coconut oil and emulsions of these oils were added to the prepared samples. No sweetening agent, salt or extra oil was added to the formulation. The formulations of peanut butters are given in Table 1.

Table 1. Peanut butter formulations

Sample code	Explanation
Con	Peanut butter without any additive (Control)
O	Peanut butter with 4% (v / w) orange oil
OCo	Peanut butter with 4% (v / w) coconut oil
Co	Peanut butter with 2% (v / w) orange oil + 2% (v / w) coconut oil
UO	Peanut butter including 4% ultrasonicated oil phase (100: 0 orange oil: coconut)
UOCo	Peanut butter including 4% ultrasonicated oil phase (50: 50 orange oil: coconut)
UCo	Peanut butter including 4% ultrasonicated oil phase (0:100 orange oil: coconut)

### Proximate composition analyses

Moisture determination in peanut butter was carried out by oven drying according to AOAC standard method (AOAC, 1990). Analyses were performed quadruplicate for each sample. The fat content of the samples was determined by the Soxhlet extractor using hexane as a solvent (Vural and Öztan, 1996). Protein (%) amounts of the products were determined using Kjeldahl apparatus (Behr Labor-Technik S3, Dusseldorf, Germany) (AOAC, 1990).

### TBARS analysis

The TBARS (2-thiobarbituric acid reactive substances) assay was performed on days 0, 6, 13 and 20 to determine malondialdehyde formation.

In the method, 5 g of the sample was homogenized with 15 ml of deionized water. 1 ml of homogenate was transferred to the test tube and 50  $\mu$ l of butylated hydroxytoluene (7.2 g/100 g) and 2 ml of thiobarbituric acid (TBA) - trichloroacetic acid (TCA) (15 mM TBA-15 g/100 g TCA) were added. The mixture was stirred with a vortex mixer and then incubated for 15 minutes in a boiling water bath for colour formation. The samples were then cooled in cold water for 10 minutes, vortexed again, and centrifuged at 2500xg for 15 minutes. The absorbance of the supernatant solution was determined at 531 nm. TBARS values are given in milligrams of malondialdehyde per kilogram

sample (Mohamed and Mansour 2012). Four repetitions were conducted for each sample.

#### pH determination

In pH measurements, 1g sample was homogenized with 9 ml of distilled water and readings were performed by immersion of the pH-meter electrode which was previously calibrated with buffer solutions. Measurements were repeated four times for each sample and the average of the results were given (Vural and Öztan, 1996).

#### Colour analysis

The CIE colour values (L\*: lightness, a\*: redness or greenness, b\*: yellowness or blueness) of the peanut butter were determined using a chroma meter (Konica Minolta, CR-400, Japan) on days 0, 6, 13 and 20 during the storage period. The instrument was calibrated with a white base supplied by the manufacturer and readings were made from four randomly selected surfaces of the sample.

#### Statistical analysis

One-way ANOVA method (Minitab 16 package program, Minitab LLC, State College, Pennsylvania, USA) was used for statistical evaluation of differences between samples and changes occurred in samples during storage period. Tukey multiple comparison test was applied to the significant variables. In addition, Pearson correlation analysis was conducted between total phenolic content and antioxidant activity results.

## RESULTS AND DISCUSSION

The mean particle sizes of the ultrasonicated emulsions of orange essential oil, coconut oil and their 1:1 mixture in sodium caseinate are given in Table 2. The emulsion containing merely orange oil had the smallest particles ( $P < 0.05$ ). Nanoemulsions did not form, because the particle size of the emulsions could not be reduced to nano level, probably due to the presence of casein in the medium.

Total phenolic and antioxidant capacity values of emulsions containing different kinds of oils prepared by ultrasonication are given in Table 3. The total phenolic content of emulsion with orange oil was the highest, and the one with coconut oil was the lowest ( $P < 0.05$ ).

Table 2. Particle sizes of orange oil, coconut oil and mixture of orange oil and coconut oil (1: 1) which were emulsified by ultrasonication

Sample code	Particle size (nm)
UO	613.0 <sup>c</sup> ± 32.3
UOCo	2252.5 <sup>a</sup> ± 159.2
UCo	1667.2 <sup>b</sup> ± 51.5

<sup>a-c</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )

UO: Orange oil emulsified by ultrasonication

UOCo: Mixture of orange oil and coconut oil (1: 1) emulsified by ultrasonication

UCo: Coconut oil emulsified by ultrasonication

Table 3. Total phenolic content and antioxidant capacity values of orange oil, coconut oil and mixture of orange oil and coconut oil (1: 1) which were emulsified by ultrasonication

Sample	Total phenolic content (mg gallic acid/ml sample)	Antioxidant capacity (DPPH) (%)	Antioxidant capacity (ABTS) (%)
UO	1.02 <sup>a</sup> ± 0.16	81.51 <sup>a</sup> ± 1.31	81.92 <sup>a</sup> ± 0.13
UOCo	0.67 <sup>b</sup> ± 0.10	77.61 <sup>a</sup> ± 4.35	38.71 <sup>b</sup> ± 2.87
UCo	0.31 <sup>c</sup> ± 0.02	49.38 <sup>b</sup> ± 3.89	21.25 <sup>c</sup> ± 1.62

<sup>a-c</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )

UO: Orange oil emulsified by ultrasonication

UOCo: Mixture of orange oil and coconut oil (1: 1) emulsified by ultrasonication

UCo: Coconut oil emulsified by ultrasonication

Antioxidant capacity analyses of emulsions in the study were carried out by the DPPH and ABTS radical scavenging activity assays. According to the results of DPPH assay, the antioxidant capacities of the emulsion containing orange oil and emulsion containing both orange and coconut oils were similar ( $P > 0.05$ ), but these emulsions had a higher antioxidant capacity than the emulsion with coconut oil ( $P < 0.05$ ). The strong antioxidant properties of orange oil, especially thanks to the limonene, might be responsible for this result. In the ABTS assay, it was found that the casein emulsion containing orange oil had the highest antioxidant capacity while the emulsion prepared with coconut oil had the lowest ( $P < 0.05$ ). As a result of three assays, total phenolic content and antioxidant capacity experiments showed similar changes among the samples, which orange oil provided high antioxidant feature to the samples.

When Pearson correlation analysis was conducted between the different antioxidant activity values,

high positive and significant correlations were found ( $r > 0.82$ ,  $P < 0.05$ ). In addition, total phenolic content was positively correlated ( $r > 0.88$ ,  $P < 0.05$ ) with both antioxidant activity test results.

Proximate analysis results of the peanut butters are given in Table 4. While the moisture content of the samples containing ultrasonicated emulsions (UO, UOCo and UCo) was found to be in the range of 10.89-12.74%, values of the other samples including different oils were between 2.32 and 4.48%. The mean moisture content of the control sample was 2.41%. The moisture contents of the emulsion added samples were higher than the other samples ( $P < 0.05$ ) due to the presence of water in the emulsions. It was observed that the emulsion added peanut butter samples had a harder structure. It was obviously seen that the sodium caseinate in the emulsion content provided a thickening property to the structure.

Table 4. Moisture (%), fat (%) and protein (%) contents of peanut butters

Sample code	Moisture (%)	Fat (%)	Protein (%)
Con	2.41 <sup>f</sup> ± 0.02	53.64 <sup>a</sup> ± 0.77	25.41 <sup>b</sup> ± 0.08
O	4.48 <sup>d</sup> ± 0.03	54.32 <sup>a</sup> ± 1.93	24.36 <sup>bc</sup> ± 0.20
OCo	3.25 <sup>e</sup> ± 0.03	55.93 <sup>a</sup> ± 0.58	22.71 <sup>d</sup> ± 0.22
Co	2.32 <sup>f</sup> ± 0.03	54.75 <sup>a</sup> ± 2.07	23.93 <sup>cd</sup> ± 0.53
UO	12.74 <sup>a</sup> ± 0.19	43.75 <sup>b</sup> ± 1.14	31.19 <sup>a</sup> ± 0.51
UOCo	11.99 <sup>b</sup> ± 0.17	46.96 <sup>b</sup> ± 0.66	29.98 <sup>a</sup> ± 0.26
UCo	10.89 <sup>c</sup> ± 0.08	46.13 <sup>b</sup> ± 0.37	30.80 <sup>a</sup> ± 0.43

<sup>a-f</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )

Insignificant differences were found among the oil content of the control and the samples with orange oil, coconut oil and the mixture of these oils ( $P > 0.05$ ). The oil content of the emulsions prepared by ultrasonication (UO, UOCo and UCo) was lower than the other samples ( $P < 0.05$ ). The water and sodium caseinate in the emulsion seemed to increase the total amount of the

product, so the amount of oil in the emulsion added samples were found to be lower.

UO, UOCo and UCo samples had a higher protein content (%) than the other samples ( $P < 0.05$ ). Sodium caseinate has a protein based structure and the addition of emulsions containing sodium caseinate is more likely to

increase the protein content of the samples. The protein (%) contents of OCo and Co samples were significantly lower than the control sample ( $P < 0.05$ ). Oils added to these samples might replace peanut tissues, resulting in a slight reduction in the amount of protein.

Peanut butter samples were stored in dark at room temperature for 20 days and pH values were determined at certain time intervals (Table 5). At the beginning of storage, the pH values of the control, peanut butter including orange oil and

the sample containing both orange and coconut oils were statistically lower than the other samples ( $P < 0.05$ ). On the last day of storage, significant differences were found among the pH values of the samples and peanut butter containing coconut oil had the highest pH value among the samples ( $P < 0.05$ ). The pH values of Con, O, OCo, UO, UOCo and UCo samples at the end of storage (day 20) were lower than those measured at the beginning of storage (day 0) ( $P < 0.05$ ).

Table 5. pH values of peanut butters during storage at room temperature

Sample code	Storage time (day)			
	0	6	13	20
Con	6.79 <sup>cA</sup> ± 0.02	6.73 <sup>bAB</sup> ± 0.06	6.75 <sup>cAB</sup> ± 0.01	6.69 <sup>dB</sup> ± 0.02
O	6.80 <sup>cA</sup> ± 0.01	6.79 <sup>abA</sup> ± 0.03	6.78 <sup>bcA</sup> ± 0.01	6.73 <sup>bcB</sup> ± 0.01
OCo	6.79 <sup>cAB</sup> ± 0.00	6.81 <sup>abA</sup> ± 0.04	6.76 <sup>bcB</sup> ± 0.00	6.72 <sup>bcdC</sup> ± 0.00
Co	6.84 <sup>bAB</sup> ± 0.00	6.87 <sup>aA</sup> ± 0.04	6.84 <sup>aAB</sup> ± 0.03	6.80 <sup>aB</sup> ± 0.03
UO	6.83 <sup>bA</sup> ± 0.02	6.78 <sup>abB</sup> ± 0.02	6.77 <sup>bcB</sup> ± 0.01	6.71 <sup>bcdC</sup> ± 0.01
UOCo	6.85 <sup>abA</sup> ± 0.00	6.83 <sup>aA</sup> ± 0.05	6.81 <sup>abA</sup> ± 0.02	6.70 <sup>cdB</sup> ± 0.01
UCo	6.87 <sup>aA</sup> ± 0.01	6.83 <sup>aA</sup> ± 0.01	6.66 <sup>dC</sup> ± 0.04	6.74 <sup>bB</sup> ± 0.01

<sup>a-d</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )

<sup>A-C</sup> Means in the same row with different superscript letters are significantly different ( $P < 0.05$ )

The lipid oxidation levels of peanut butter samples stored in the dark at room temperature during 20 days of storage were determined by TBARS analysis, and the results are presented in Table 6. Significant differences were found among TBARS values of samples at the beginning of storage ( $P < 0.05$ ). On the last day of storage (day 20), however, TBARS values of the emulsified samples (UO, UOCo and UCo) prepared by ultrasonication were considerably higher than those of the other samples ( $P < 0.05$ ). At the end of storage, mold growth was observed in these three emulsion added samples and therefore storage was terminated. The high moisture values of the emulsion added samples and the formation of microbial growth in these samples might trigger lipid oxidation rate. Excluding the samples that the emulsions were

added, TBARS values of the samples decreased at the end of storage compared to the initial values ( $P < 0.05$ ). This might be due to the antioxidant substances found in oils added to peanut butter. El-Rawas et. al., (2012) examined the effects of electron beam irradiation on quality of peanut butter. TBARS values of the non-irradiated sample were 0.98 and 1.61 mg malondialdehyde per kilogram sample on the 2<sup>nd</sup> and 14<sup>th</sup> days of storage respectively.

CIE colour values measured during storage ( $L^*$ ,  $a^*$ ,  $b^*$ ) are given in Table 7.  $L^*$  values were lower in the samples (UO, UOCo and UCo) with emulsions prepared by ultrasonication at all storage times (days 0, 6, 13 and 20) ( $P < 0.05$ ). It was observed that, with naked eye, the colour of the emulsified samples was darker than the other

samples. The L\* values obtained by the instrument were compatible with the sensory perceived colour of the samples. Significant differences were found among the samples in terms of redness (a\*) and yellowness (b\*) at all times of storage ( $P < 0.05$ ). El-Rawas et. al., (2012) found the L\*, a\* and b\* values of the untreated peanut butter 57.56, 11.22 and 29.50 respectively on the 2<sup>nd</sup> day of the storage. Norazatul Hanim et.

al., (2016) found that increasing grinding time reduced the L\*value of peanut butter. They stated that the void space between the particles decreased by obtaining finer particle size and this increased the absorption of the light. Çiftçi et. al., (2008) reported that longer grinding time caused heating which accelerates browning reactions.

Table 6. TBARS values of (mg malondialdehyde/kg sample) peanut butters during storage at room temperature

Sample code	Storage time (day)			
	0	6	13	20
Con	0.30 <sup>aA</sup> ± 0.11	0.25 <sup>aAB</sup> ± 0.07	0.07 <sup>aC</sup> ± 0.01	0.14 <sup>bBC</sup> ± 0.00
O	0.21 <sup>abA</sup> ± 0.02	0.19 <sup>abA</sup> ± 0.00	0.04 <sup>abcB</sup> ± 0.02	0.07 <sup>bcB</sup> ± 0.05
OCo	0.23 <sup>abA</sup> ± 0.04	0.18 <sup>bA</sup> ± 0.00	0.06 <sup>abB</sup> ± 0.02	0.05 <sup>cB</sup> ± 0.01
Co	0.14 <sup>bcB</sup> ± 0.05	0.20 <sup>abA</sup> ± 0.01	0.03 <sup>bcdC</sup> ± 0.01	0.06 <sup>bcC</sup> ± 0.01
UO	0.25 <sup>abAB</sup> ± 0.11	0.15 <sup>bB</sup> ± 0.01	0.01 <sup>dC</sup> ± 0.01	0.34 <sup>aA</sup> ± 0.02
UOCo	0.05 <sup>cC</sup> ± 0.01	0.14 <sup>bB</sup> ± 0.02	0.02 <sup>cdC</sup> ± 0.02	0.37 <sup>aA</sup> ± 0.01
UCo	0.05 <sup>cC</sup> ± 0.01	0.16 <sup>bB</sup> ± 0.02	0.04 <sup>bcdC</sup> ± 0.01	0.38 <sup>aA</sup> ± 0.07

<sup>a-d</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )

<sup>A-C</sup> Means in the same row with different superscript letters are significantly different ( $P < 0.05$ )

Table 7. Colour values of peanut butters during storage at room temperature

Colour parameter	Day	Sample code						
		Con	O	OCo	Co	UO	UOCo	UCo
L*	0	70.92 <sup>aA</sup>	71.74 <sup>aA</sup>	72.56 <sup>aA</sup>	70.89 <sup>aA</sup>	54.68 <sup>bA</sup>	57.57 <sup>bA</sup>	59.12 <sup>bA</sup>
	6	65.79 <sup>bA</sup>	70.61 <sup>aA</sup>	69.99 <sup>aB</sup>	70.01 <sup>aA</sup>	47.90 <sup>dC</sup>	54.76 <sup>cAB</sup>	55.91 <sup>cBC</sup>
	13	67.82 <sup>aA</sup>	70.75 <sup>aA</sup>	70.05 <sup>aB</sup>	71.18 <sup>aA</sup>	48.60 <sup>bBC</sup>	45.96 <sup>bB</sup>	54.28 <sup>bC</sup>
	20	69.10 <sup>aA</sup>	71.61 <sup>aA</sup>	70.52 <sup>aB</sup>	70.07 <sup>aA</sup>	51.09 <sup>cB</sup>	51.40 <sup>cAB</sup>	56.82 <sup>bB</sup>
a*	0	1.98 <sup>bB</sup>	2.05 <sup>bA</sup>	2.64 <sup>abA</sup>	2.85 <sup>abA</sup>	2.38 <sup>abB</sup>	2.93 <sup>abB</sup>	3.18 <sup>aA</sup>
	6	4.00 <sup>aA</sup>	2.44 <sup>cA</sup>	2.94 <sup>bcA</sup>	2.27 <sup>cdA</sup>	3.32 <sup>abA</sup>	1.58 <sup>deD</sup>	1.27 <sup>cC</sup>
	13	3.70 <sup>aA</sup>	2.54 <sup>cdA</sup>	2.97 <sup>bcA</sup>	2.62 <sup>cdA</sup>	3.46 <sup>abA</sup>	2.30 <sup>dC</sup>	2.34 <sup>dB</sup>
	20	2.05 <sup>cB</sup>	2.17 <sup>cA</sup>	2.87 <sup>abcA</sup>	2.48 <sup>bcA</sup>	3.77 <sup>aA</sup>	3.54 <sup>abA</sup>	3.42 <sup>abA</sup>
b*	0	26.29 <sup>bA</sup>	27.41 <sup>abA</sup>	27.17 <sup>abB</sup>	28.18 <sup>abA</sup>	26.30 <sup>bA</sup>	29.53 <sup>abA</sup>	30.41 <sup>aA</sup>
	6	31.06 <sup>aA</sup>	26.86 <sup>abA</sup>	28.37 <sup>abA</sup>	27.63 <sup>abA</sup>	26.96 <sup>abA</sup>	24.47 <sup>bD</sup>	23.47 <sup>bC</sup>
	13	27.92 <sup>aA</sup>	28.52 <sup>aA</sup>	27.91 <sup>aAB</sup>	27.86 <sup>aA</sup>	26.36 <sup>bA</sup>	25.86 <sup>bC</sup>	26.33 <sup>bB</sup>
	20	25.44 <sup>cA</sup>	27.98 <sup>bA</sup>	27.99 <sup>bAB</sup>	28.31 <sup>bA</sup>	27.12 <sup>bcA</sup>	28.24 <sup>bB</sup>	30.56 <sup>aA</sup>

<sup>a-e</sup> Means in the same row with different superscript letters are significantly different ( $P < 0.05$ )

<sup>A-D</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ )



As the L\* values of the samples were examined in terms of storage time, Con, O and Co samples showed no change during storage ( $P > 0.05$ ), whereas the L\* values of OCo sample and emulsified samples (UO, UOCo and UCo) tended to decrease over time ( $P < 0.05$ ). Changes were observed in a\* values of control and the samples including ultrasonicated oils ( $P < 0.05$ ) over time, while no change was observed in the other samples ( $P > 0.05$ ). When the b\* values at the beginning of storage were compared with the values at the end of storage, just the b\* value of UOCo at the end of storage was found to be significantly different from its initial value ( $P < 0.05$ ), but for the other samples, no difference was observed ( $P > 0.05$ ).

As a result, the addition of ultrasonicated emulsions containing orange oil, orange oil-coconut oil mixture and coconut oil into peanut butter reduced the sensitivity to lipid oxidation for about two weeks. However, further study is needed to produce nano-size emulsions, which may lead to more effective results.

#### ACKNOWLEDGEMENTS

Authors thank to TUBITAK (The Scientific and Technological Research Council of Turkey) for the financial support of the project with the Scholarship "2209-A University Students Research Projects Support Program".

#### REFERENCES

- Abbaszadeh, S., Sharifzadeh, A., Shokri, H., Khosravi, A.R., Abbaszadeh, A. (2014). Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. *J Mycol Med*, 24(2): e51-e56.
- Anton, A.A., Fulcher, R.G., Arntfield, S.D. (2009). Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: effects of bean addition and extrusion cooking. *Food Chem*, 113: 989-996.
- Anton, N., Vandamme, T.F. (2009). The universality of low-energy nano-emulsification. *Int J Pharm*, 377(1-2): 142-147.
- AOAC. (1990). Official methods of analysis of the association of official analytical chemists. Washington, USA: Association of Official Analytical Chemists.
- Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol*, 28: 25-30.
- Canselier, J.P., Delmas, H., Wilhelm, A.M., Abismail, B. (2002). Ultrasound emulsification-an overview. *J Dispers Sci Technol*, 23: 333-349.
- Carraeu, P.J., Cotton, F., Citerne, G.P., Moan, M. (2002). Rheological properties of concentrated suspensions: applications in foodstuffs. In: *Engineering and food for the 21st century*, Welti-Chanes, J., Barbosa-Canovas, G.V., Aguilera, J.M. (eds.), CRC Press, New York, pp. 327-345.
- Çiftçi, D., Kahyaoglu, T., Kapucu, S., Kaya, S. (2008). Colloidal stability and rheological properties of sesame paste. *J Food Eng*, 87: 428-435.
- Co, E., Marangoni, A.G. (2012). Organogels: an alternative edible oil- structuring method. *J Am Oil Chem Soc*, 89(5): 749-780.
- Cucheval, A., Chow, R.C.Y. (2008). A study on the emulsification of oil by power ultrasound. *Ultrason Sonochem*, 15: 916-920.
- Donsi, F., Annunziata, M., Sessa, M., Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT-Food Sci Technol*, 44(9): 1908-1914.
- El-Rawas, A., Hvizdzak, A., Davenport, M., Beamer, S., Jaczynski, J., Matak, K. (2012). Effect of electron beam irradiation on quality indicators of peanut butter over a storage period. *Food Chem*, 133: 212-219.
- Ghasemi, S., Jafari, S.M., Assadpour, E., Khomeiri, M. (2017). Production of pectin-whey protein nano-complexes as carriers of orange peel oil. *Carbohydr Polym*, 177: 369-377.
- Gogate, P.R., Tayal, R.K., Pandit, A.B. (2006). Cavitation: a technology on the horizon. *Curr Sci (Bangalore)*, 91: 35-46.

- Hashtjin, A.M., Abbasi, S. (2015). Nano-emulsification of orange peel essential oil using sonication and native gums. *Food Hydrocoll*, 44: 40-48.
- Huang, Q., Yu, H., Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *J Food Sci*, 75(1): R50–R57.
- İlyasoğlu, H., El. S.N. (2010). Nanoemülsiyonlar: Oluşumları, yapıları ve kolloidal salınım sistemleri olarak gıda sektöründe kullanım alanları. *GIDA*, 35(2): 143-150.
- Jayadas N.H., Nair K.P. (2006). Coconut oil as base oil for industrial lubricants-evaluation and modification of thermal, oxidative and low temperature properties. *Tribol Int*, 39: 873-878.
- Lu, W.-C., Huang, D.-W., Wang, C.-C.R., Yeh, C.-H. Tsai, J.-C. Huang, Y.-T. Li, P.-H. (2018). Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. *J Food Drug Anal*, 26: 82-86.
- Martins, P.F., Medeiros, H.H.R., Sbaite, P., Wolf Maciel, M.R. (2013). Enrichment of oxyterpenes from orange oil by short path evaporation. *Sep Purif Technol*, 116: 385-390.
- McClements, D.J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter*, 7: 2297-2316.
- McClements, D.J., Decker, E.A., Park, Y., Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Crit Rev Food Sci Nutr*, 49(6): 577-606.
- Mohamed, H.M.H., Mansour, H.A. (2012). Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. *LWT-Food Sci Technol*, 45: 79-87.
- Norazatul Hanim, M.R., Chin, N.L., Yusof, Y.A. (2016). Effects of grinding time on rheological, textural and physical properties of natural peanut butter stored at different temperatures. *J Texture Stud*, 47: 131-141.
- Özogul, Y., Durmus, M., Ucar, Y., Özogul, F., Regenstein, J.M. (2016). Comparative study of nanoemulsions based on commercial oils (sunflower, canola, corn, olive, soybean, and hazelnut oils): Effect on microbial, sensory, and chemical qualities of refrigerated farmed sea bass. *Innov Food Sci Emerg*, 33: 422-430.
- Pengon, S., Chinatangkul, N., Limmatvapirat, C., Limmatvapirat, S. (2018). The effect of surfactant on the physical properties of coconut oil nanoemulsions. *Asian J. Pharm*, 13: 409-414.
- Piorkowski, D.T., McClements, D.J. (2013). Beverage emulsions: Recent developments in formulation, production, and applications. *Food Hydrocoll*, 42: 5-41.
- Ramisetty, K.A., Pandit, A.B., Gogate, P.R. (2015). Ultrasound assisted preparation of emulsion of coconut oil in water: Understanding the effect of operating parameters and comparison of reactor designs. *Chem Eng Process*, 88: 70-77.
- Rodriguez, M.V., Medina, L.M., Jordano, R. (2002). Prolongation of shelf life of sponge cakes using modified atmosphere packaging. *Acta Aliment*, 31: 191-196.
- Sanders III, C.T., DeMasie, C.L., Kerr, W.L., Hargrove, J.L., Pegg, R.B., Swanson, R.B. (2014). Peanut skins-fortified peanut butters: Effects on consumer acceptability and quality characteristics. *LWT-Food Sci Technol*, 59: 222-228.
- Santos, K.A., Filho, O.P.A., Aguiar, C.M., Milinsk, M.C., Sampaio, S.C., Palú, F., Antônio da Silva E., (2017). Chemical composition, antioxidant activity and thermal analysis of oil extracted from favela (*Cnidocolus quercifolius*) seeds. *Ind Crop Prod*, 97: 368-373.
- Sivakumar, M., Tang, S.Y., Tan, K.W. (2014). Cavitation technology – a greener processing technique for the generation of pharmaceutical nanoemulsions. *Ultrason Sonochem*, 21: 2069-2083.
- Tan K.W., Tang S.Y., Thomas, R., Vasanthakumari, N., Manickam, S. (2016). Curcumin-loaded sterically stabilized nanodispersion based on non-ionic colloidal

system induced by ultrasound and solvent diffusion-evaporation. *Pure Appl Chem*, 88: 43-60.

Tanti, R., Barbut, S., Marangoni, A.G. (2016). Oil stabilization of natural peanut butter using food grade polymers. *Food Hydrocoll*, 61: 399-408.

Vieira, A.J., Beserra, F.P., Souza, M.C., Totti, B.M., Rozza, A.L. (2018). Limonene: Aroma of innovation in health and disease. *Chem-Biol Interact*, 283: 97-106.

Vural, H., Öztan, A. (1996). *Et ve Ürünleri Kalite Kontrol Laboratuvarı Uygulama Kılavuzu*. Hacettepe Üniversitesi Mühendislik Fakültesi Yayınları No: 36, Ankara, Turkey, 236 p.