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Determination of Some Quality Characteristics and Rheological Properties of Yoghurts Made Using Cow Milk and Soy Drink Mixture Enriched with Pomegranate Peel Extract

Hasan TEMİZ*, Elif Büşra ERSÖZ

Department of Food Engineering, Engineering Faculty, Ondokuz Mayis University, Samsun, Turkiye

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Corresponding Author: Hasan TEMİZ, E-mail: hasant@omu.edu.tr
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ABSTRACT

This study examined the applicability of pomegranate peel extracts (PPE) and microencapsulated PPE extracts (MPE) in yoghurts which are made with cow's milk and soy drink mixtures. For preparing PPE, pomegranate peel powders were extracted by 50% ethanol in an ultrasonic water bath. PPE was encapsulated by using a spray dryer. Phytochemical composition and antioxidant activity of PPE and MPE were determined and PPE and MPE were added to cow milk and soy drink mixture (4:1) for yoghurt production at 0.5% and 1% rates, respectively. Physicochemical, rheological, microbial and sensory properties of the yoghurt samples stored at 4 °C were determined during storage. Extract addition affected storage modulus (G') values and lost tangent (tanδ) values. The extract additive prevented the growth of yeasts and moulds and extended the shelf life of the samples. The favorable effect of the extract on taste and aroma was determined in sensory tests. The overall acceptability scores for PY1 and MY1 samples increased during the storage time and the higher scores was determined on the 28th days of storage. However, the extract contributed to the loss of textural properties such as syneresis and visible viscosity.

Keywords: Yoghurt, Ultrasound, Microencapsulation, Antioxidant, Rheology, Sensory

1. Introduction

Functional foods are defined as food or food ingredients that provide additional benefits to human physiology and metabolic functions, beyond providing basic nutritional requirements of the body, thereby preventing diseases and achieving a healthier life (Kotilainen et al. 2006). Most of the agri-food industry wastes are composed of bioactive polyphenolic phytochemicals and these waste products have the potential to become significant functional food (Amyrgialaki et al. 2014).

Pomegranate (*Punica granatum* L.), which originates from Iran, is known as one of the health-beneficial fruits with its phenolic component content (Akhtar et al. 2015; Kazemi et al. 2016). Pomegranate peel, is a valuable waste of pomegranate juice production, constitutes 26-30% of the total weight of the fruit and contains large amounts of important phenolic compounds such as flavonoids and hydrolysable tannins (Ismail et al. 2012). Due to its rich amounts of vitamins, polysaccharides, polyphenols and minerals, pomegranate peel is used in many countries such as India and Egypt in the field of ethnopharmacology especially in the treatment of diseases such as diarrhea, dysentery and dental plaque (Tripathi et al. 2014). Pomegranate peel shows higher antioxidant activity, phenolic component and therefore bioavailability compared to pomegranate fruit and it can be used as a nutritional supplement or functional input in food formulations (Surek & Nilufer-Erdil 2016). Researchers suggest that pomegranate peels should be used as low-cost nutritional supplements in the low-income countries rather than wasted in the environment (Gullon et al. 2016).

The evaluation of plant-derived extracts as food additives has been a popular subject for many studies (Caleja et al. 2016; Çam et al. 2014; Karaaslan et al. 2014). But plant extracts cannot show the desired stability in food processes and storage (Robert et al.

2010). The stabilization of extracts rich in phenolic compounds by microencapsulation and constituting the desired properties of food systems also has been the subject of many studies (Çam et al. 2014; Kaderides et al. 2015; Robert et al. 2010).

Soy drink is widely consumed especially in Far East countries and it is equivalent to cow's milk in terms of many essential nutrients. In addition to being a functional nutrient, lactose intolerance, milk protein allergy, and vegetarian diet requirements also increase the interest in this product. Although soy drinks are widely consumed in Far Eastern countries sensory problems limited the use of this products in societies that soy-based nutrition is not widespread (Trindade et al. 2001; Vij et al. 2011; Wang et al. 2003).

Soy yoghurt produced from soy drink cannot provide the expected sensory and physicochemical properties due to its chemical composition (Gu et al. 2015). To eliminate these negative properties many methods such as mixing with cow's milk (Temiz & Çakmak 2018), using aromatic herbal sources (Ye et al. 2013), stabilizers (Cho et al. 2013) and new technologies (Ferragut et al. 2009) have been tried.

In this study, we aimed to develop a new product with the use of pomegranate industry wastes that has an important functional composition. The primary aim of the study is to evaluate the pomegranate peel, which has numerous benefits to health, as a food additive and to present it to human nutrition. The pomegranate peel extract (PPE) is microencapsulated to increase the stability and functional properties of the extract during food processing and storage. By using microencapsulated PPE (MPE) and liquid PPE as a functional ingredient in yoghurt made with cow milk and soy drink mixture, it is aimed to eliminate the bad aroma based on soy and increase the functional properties of yoghurts.

2. Material and Methods

2.1. Materials

Pomegranate peels and soybeans obtained from the local market were used in this study. Without damaging the peels, the fruit part and the peel part were separated and the peels were stored at -18 °C until use. Soy drink production was carried out according to the method described by Temiz & Çakmak (2018). The dry matter of soybean drink produced is 12.42%, the fat content is 3.2%, protein content is 5.8%, and the pH is 6.72. Raw cow milk (dry matter 10.23%, fat 3.1%, protein 4.63% and pH 6.74) was obtained from the dairy enterprise operating in region. Mixed starter culture Y 410 500 I: *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus* (Maysa Gıda San. Tic. AŞ. İstanbul, Türkiye) was used in the production of yoghurt samples. Maltodextrin having DE of 13-17 was obtained from Aldrich Company (St. Louis, MO, USA). The rest of the chemicals and standards were analytical grade and procured from Sigma or Merck (Darmstadt, Germany).

2.2. Extraction of pomegranate peels

To prepare PPE, dried peel powders were extracted by 50% ethanol at a solvent to peel powder ratio of 4:1 (v/w) in an ultrasonic water bath (Isolab Laborgerate GmbH, Germany) for 30 minutes at 4 °C. The temperature control of the ultrasonic water bath was made by the water circulation at 0 ºC. The extract was centrifuged at 10,000*xg* for 15 minutes at 4 °C (Hettich Zentrifugen Universal 320 R, Germany) and the supernatant was separated. The solvent was evaporated under 40 ºC using a rotary evaporator (Buchi Rota Vapor K-3, Buchi, Switzerland). The concentrated PPE (Brix 30) was stored at 4 ºC until analysis (Kaderides et al. 2015).

2.3. Microencapsulation of extract

PPE was microencapsulated by a spray drying technique using maltodextrin (DE 13-17) as the coating material. The coating solution that used in the microencapsulation process was prepared according to the method described by Çilek et al. (2012). After the preparation, the coating material was mixed with PPE (4:1 v/v) and homogenized for 10 minutes (IKA-Werke GmbH & Co. KG, Staufen, Germany). Drying was carried out in a laboratory-scale spray dryer (Buchi Mini Spray Dryer B290, Switzerland). The drying process was carried out according to Çam et al. (2014) method with slight modification. For the production of MPE, process conditions were determined as air inlet temperature at 160±5 ºC and outlet temperature 70±2 ºC, extract coating material ratio 1:4 (v/v) and solid feed ratio 30% (w/w).

2.4. Spectrophotometric analysis

MPE was extracted for spectrophotometric analysis. 0.2 g MPE was dissolved with 20 mL methanol: acetic acid: water (50:8:42 v/v/v). The mixture was vortexed for 1 minute and then incubated twice at 4 ºC for 20 minutes in an ultrasonic water bath (Isolab Laborgerate GmbH). The supernatant was centrifuged at 12,000*x g* for 5 minutes (Hettich Zentrifugen Universal 320 R, Germany), then filtered

and stored at 4 °C until analysis was performed (Robert et al. 2010). The total phenolic content (TPC) was determined according to the Folin-Ciocalteu colorimetric method (Fawole & Opara 2016). The calibration curve (R^2=0.9967) was determined using different gallic acid concentrations (20, 40, 60, 80, 100 ppm). TPC of PPE was expressed as gallic acid equivalent (GAE) in milligrams per mL. TPC of MPE was expressed as GAE in milligrams per g.

For surface phenolic content (SPC) of MPE, Robert et al. (2010) method was applied with little modification. 0.5 g of MPE and 25 mL of ethanol: methanol (1:1) were vortexed. The mixture was centrifuged at 3500 rpm for 3 minutes (Hettich Zentrifugen Universal 320 R, Germany) and the SPC in the clear portion was determined according to the Folin-Ciocalteu colorimetric method (Fawole & Opara 2016).

Total flavonoid concentration (TFC) was determined as described by (Fawole & Opara 2016), and the results were expressed as catechin equivalents (CE) per mL (g) sample. The results were calculated according to the catechin standard curve (R^2=0.9997). Total anthocyanin content (TAC) was measured as described by El Kar et al. (2011), and the results were expressed as mg cyaniding 3-O-glucoside per 1,000 mL (g) of the sample.

The antioxidant activity was evaluated with the scavenging method of DPPH as reported by Kazemi et al. (2016). The extract solutions were allowed to stay in the dark for 30 minutes at room temperature and then their absorbance at 517 nm was read by spectrophotometer. Two parallel studies were performed for each solution. Different concentrations of Trolox solutions were used to create linear regression equations (R^2=0.9927). Results were given in Trolox equivalents (TE) per 100 mL of extract. Antioxidant activity of the samples was also measured using the $ABTS^+$ radical cation capture activity method described by Mushtaq et al. (2015). Different concentrations of Trolox solutions were used to create linear regression equations (R^2=0.9927). Results were given in TE per 100 mL of extract. All spectrophotometric measurements were conducted in duplicate.

2.5. Yield and efficiency of microencapsulation

The yield and efficiency of the microencapsulation process were determined by the calculation method stated by Kaderides et al. (2015). The following equations are used for the yield and efficiency of the microencapsulation process.

$$\text{efficiency}=[(\text{TPC-SPC})/\text{TPC}]\times 100 \quad (1)$$

$$\text{yield}=(\text{total weight of microencapsules/total weight of input})\times 100 \quad (2)$$

2.6. Yoghurt production

Yoghurt production was carried out as stated in our previous study (Temiz & Çakmak 2018) in the Ondokuz Mayıs University Faculty of Agriculture Milk Processing Plant. As a result of the preliminary experiments and literature knowledge the mixing milk ratios to be used in the production of yoghurts were determined as 4:1 cow milk:soy drink. Yoghurt milk mixture was heat-treated at 85±2 °C for 20 minutes. After cooling the yoghurt milk to 65 °C, the mixture was added to 0% (control sample, CC0), 0.5%, 1% PPE (PY1 and PY2, respectively) and MPE (MY1 and MY2, respectively). MPE was added to the milk to make it equal to the TFC of the PPE. Yoghurt samples were stored for 28 days at 4 ºC for analysing and analyses were made on the 1st, 7th, 14th, 21st and 28th days of the storage periods. But, the 28th day analyses of the control yoghurts, it was not conducted due to the signs of mold.

2.7. Physicochemical analysis

The pH of the samples was determined by using a digital pH meter (Cyberscan PC 510, Eutech instruments, Ayer Rajah Crescent, Singapore). The lactic acid content in the samples was measured by titration using NaOH (0.1 mol/L) and expressed in lactic acid (%). For the determination of syneresis (%), 5 g of the sample was centrifuged at 2,500*x g* for 10 min at 4 °C (Sigma Model 3K30, Osterode am Harz, Germany) and the results were expressed as % syneresis based on the percentage of the amount of supernatant in the weighed sample amount.

2.8. Rheological measurements

Rheological measurements in yoghurt were performed using parallel plates (diameter: 35 mm, gap: 1 mm) in a rheometer (HAAKE Mars III; Thermo Scientific, Germany) at 4 °C. The samples were mixed in a magnetic stirrer at 100 rpm for 1 minute before rheological tests were performed on yoghurts. For all tests, a 1 mL yoghurt sample was taken and placed between the plates and allowed to equilibrate (4 °C) for 2 minutes. Two types of tests were conducted on yoghurts. For steady sweep tests, the samples were sheared

continuously at a rate ranging from 1-100/s at a constant stress of 1 Pa and shear stress values were recorded according to shear rates in this range. Apparent viscosity value (η_{50}) was expressed to be the same as the feeling of shear in the mouth, calculated at a shear rate of 50/s viscosity value was determined (Morris 1994). To determine the dynamic viscoelastic properties, frequency sweep tests were carried out over a frequency range of 0.1 to 10 Hz at 1 Pa. The rheological parameters storage modulus (G'), loss modulus (G"), and loss tangent (tanδ) which is equal to G"/G' were determined during the test. Calculation of these measurements was performed using Rheowin 4 Data Manager software (version 4.20, Haake). All the rheological parameters were the mean of two measurements of samples.

2.9. Microbiological analysis

Ten g of yoghurt sample was mixed with 90 mL 0.1% peptone (Merck Darmstadt, Germany) water in a Stomacher. Decimal dilutions were prepared with values of 10^{-2} to 10^{-9} for each sample. The viable lactobacilli count was determined according to the pour plate method using de Man, Rogosa and Sharpe agar. *Streptococci* count was determined by the pour plate method using M17 agar. Total yeast and mould count were determined by the spreading method using yeast extract glucose chloramphenicol agar (Temiz & Dagyildiz 2018). Two replicates were performed for each sample.

2.10. Sensory evaluation

For the sensory evaluation of the samples, before sensory analysis a panel of 10 people was informed about the evaluation. Each of the yoghurt samples was coded with a different number and presented to the panelists. Sensory evaluations were performed on the 1st, 7th, 14th, 21st and 28th days of storage. Panelists were asked to rate yoghurts according to their liking. Scoring categories were color and appearance, texture, taste and aroma, and overall acceptability. In the color and appearance and texture category, the samples were evaluated by 1-5 points (5= like extremely, 1= dislike extremely) scale. In the taste and aroma category, samples were asked to be evaluated in the range of 1-9 points hedonic scale (9= like extremely, 1= dislike extremely). Overall acceptability scores are presented as the average of the scores of the samples evaluated.

2.11. Statistical analysis

Samples were analyzed using SPSS Statistical Software (2000) (SPSS Inc., Chicago, IL, USA), and the results were offered as mean ± standard deviation. Significance differences ($p<0.05$) among the different types of samples and the effect of storage time were analyzed with ANOVA, followed by Duncan's multiple range tests. All measurements were conducted in duplicate.

3. Results and Discussion

3.1. Chemical composition and antioxidant activity of PPE and MPE

TPC, TAC and TFC analysis results of PPE and MPE are given in Table 1. As seen from the Table, there were significant differences between treatments ($p<0.05$). TPC content was determined higher in the PPE samples while TAC and TFC contents were determined higher in MPE samples. TPC in PPE was calculated as 108.44 GAE/mL, TFC was 9.55 mg CE/mL and TAC was 223.43 mg Cyn-3-glu/L extract. In MPE, TPC was calculated as 105.30 GAE/mL, TFC was 16.29 mg KE/mL and TAC was 410.68 mg Cyn-3-glu/L. The majority of phenolics in PPE are gallic acid, ellagic acid, punicalin and punicalagin and other condensable tannins (Ismail et al. 2012). Surek and Nilufer-Erdil (2016) reported TPC, TFC and TAC of pomegranate peel as 18029.2 mg GAE/100 g; 21,758 mg CE/100 g and 51.8 mg Cyn-3-glu/100 g, respectively. The results are different from our study and this is due to many reasons such as extraction process and pomegranate variety. The extraction of pomegranate peel phytochemicals in methanol mixtures is more efficient (Ismail et al. 2012). Fawole and Opara (2016) calculated that TPC in the PPE in ethanol: water (1:1) mixtures was 2992.93 mg GAE/100 mL. In the same study, it was stated that the amount of phenolic substance was highest in extracts prepared in alcohol-water mixtures compared to pure ethanol (2458.03 mg GAE/100 mL) and pure water (2658.00 mg GAE/100 mL). TFC in PPE was expressed as 1505.00 mg CE/100 mL. Orak et al. (2012), the average TPC of three different types of Hicaznar peel extract is expressed as 160.70 mg GAE/g. TFC in peel was reported to be between 9.44 and 19.93 mg quercetin/g.

In other studies, TPC of pomegranate peel was reported to be 101,856 mg GAE/g (Fischer et al. 2011) and 118.2-370 mg GAE/g Amyrgialaki et al. 2014). TPC in the PPE may vary for many reasons; pomegranate species, pomegranate maturity; extraction method, duration, temperature; solvent ratio, solvent type, etc.

Radical cation reduction activity is associated with the amount of phenolic compounds in many studies (Amyrgialaki et al. 2014; Surek & Nilufer-Erdil 2016; Turgut et al. 2016). Pomegranate peel has more phenolic components than all other parts of the fruit (pulp,

seed, and leaf). Fawole and Opara (2016) stated that pomegranate extract has 5-30 times more radical reduction power than fruit pulp. Therefore, it is recommended that PPE be used for the functional ingredient in food systems (Fawole & Opara 2016; Surek & Nilufer-Erdil 2016). The evaluation of antioxidant activity by a single method is very difficult because of the complexity of the antioxidant mechanism. Additionally, many factors such as temperature, the chemical structure of phenolics, and the pH of the environment affect this mechanism. Therefore, the measurement of antioxidant activity cannot be adequately assessed by a single method (Surek & Nilufer-Erdil 2016).

The results of the antioxidant activity analysis of PPE and MPE are given in Table 1. Although there was not much decrease in antioxidant activity because of the microencapsulation process, $ABTS^+$ radical cation capture activity reduction power of both PPE and MPE was found to be higher than DPPH reduction power. Surek & Nilufer-Erdil (2016) and Fischer et al. (2011) calculated the DPPH and ABTS activities in pomegranate peels as 45099.6 mg TEAC/100 g and 51100.8 mg TEAC/100 g, respectively.

Mushtaq et al. (2015) stated that ABTS reduction power in pomegranate peels extracted by using different enzymes is between 118.25-445.02 mM TE/g. In the same study, it was stated that enzyme supported extraction was more efficient than solvent extractions, especially in $ABTS^+$ reduction power. $ABTS^+$ and DPPH reduction power of PPE determined in our study did not show much difference according to the literature, but it was lower than the enzyme-assisted extraction study.

Table 1- Chemical composition and antioxidant activity of pomegranate peel extracts and microencapsulated pomegranate peel extracts

Parameter	***PPE***	***MPE***
TPC (mg GAE/mL)	108.44 ± 0.28^a	105.30 ± 1.21^b
TFC (mg CE/mL)	9.55 ± 0.02^b	16.29 ± 0.16^a
TAC (mg Cyn-3-glu/L)	223.43 ± 3.34^b	410.68 ± 2.56^a
DPPH (TE/100 mL)	71.24 ± 0.16^a	69.90 ± 0.65^a
ABTS (TE/100 g)	100.97 ± 0.21^a	93.30 ± 0.26^b

PPE: Pomegranate peel extracts, MPE: Microencapsulated pomegranate peel extracts, TPC: Total phenolic compound, TFC: Total flavonoid content, TAC: Total anthocyanin content, DPPH: Scavenging activity of DPPH; ABTS; radical cation capture activity of ABTS. Small letters show the significant difference ($p<0.05$) between treatments. Analytical results are the means ± standard deviation of three replicates

3.2. Encapsulation efficiency and encapsulation yield

As a result of microencapsulation process analysis, microencapsulation yield and efficiency were calculated as 37.6% and 78.21%, respectively. Çam et al. (2014) investigated microencapsulation optimization of PPE in spray dryer and stated that process yield was calculated as 49.8% and process efficiency as 98.8% at 160 °C inlet temperature. The efficiency and yield of microencapsulation in the spray dryer were dependent on many different parameters. Although the inlet temperature is almost the same, it can be said that the yield and efficiency of our system were low due to the different parameters such as feed rate, outlet temperature, and coating rate. In the same study, the amount of phenolic compounds in the microcapsule at 160 °C was expressed to be 94.6 mg GAE/g and this value was similar to our study. Kaderides et al. (2015) optimized the microencapsulation of PPE with different materials, the efficiency of microencapsulation was reported to be between 69.80-99.80%. Although it depends on many other factors, it was stated that using only maltodextrin as a coating material decreased the efficiency of the process, but the protein-containing coating materials (whey powder, skimmed milk powder) were used with maltodextrin increased the efficiency of the system.

The efficiency and yield of the system in the microencapsulation technique with the spray dryer depend on many factors. Studies indicated that increasing the inlet temperature reduces the system efficiency whereas decreasing the temperature prevents effective drying. However, many parameters affect the system, such as the type of coating material and coating ratio and feed flow rate (Goula & Lazarides 2015).

3.3. First fermentation time of yoghurt

pH and time factors effects on the yoghurt formation of the milk samples are given in Figure 1. As can be seen from the Figure 1, a faster pH decrease occurred in the control sample when compared to the other samples, and the time to decrease to pH 4.71 was 120 minutes. For the extract-added samples this time was 240 minutes while the time reached to 4.71 pH value for microencapsulated samples was 270 minutes. These results show that the addition of extract and microencapsulated extract had an effect on the first fermentation period of yoghurt, and microcapsules caused to prolong the growth times of bacterial cultures. Resources have shown that pomegranate peel phenolic causes lysis of cell membrane proteins (Ismail et al. 2012; Akhtar et al. 2015).

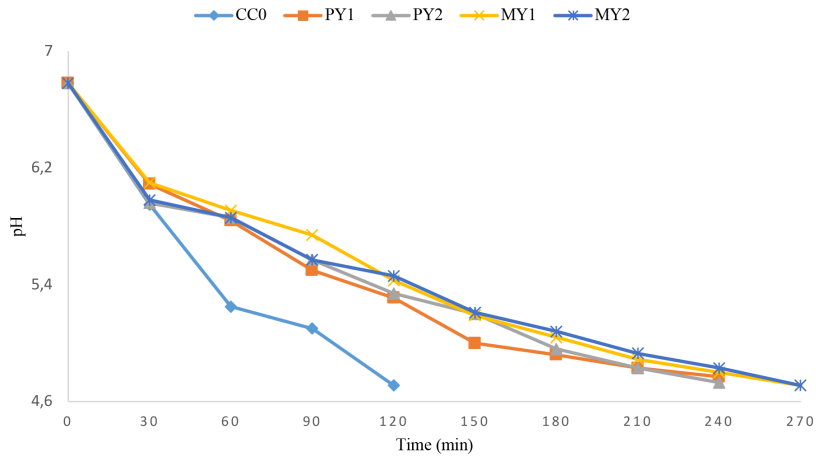

Figure 1- First fermentation time of yoghurt. CC0: control sample; PY1 and PY2: soy yogurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: soy yogurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively

3.4. Physicochemical characteristics of yoghurts

The pH values and titratable acidity (% lactic acid) values of samples during storage are given in Figure 2. The pH values of the samples varied between 4.02-4.65. In all samples, a decrease in pH values was observed during the storage time. The decrease in pH during storage in yoghurts is due to starter cultures producing organic acid by fermenting carbohydrates (Bedani et al. 2014a). It was a significant effect at 1st, 7th and 14th days of storage time ($p<0.05$) while the effect of PPE and MPE on pH change was not significant at the 1st, 21st and 28th days of the storage time ($p>0.05$). Osman and Razig (2010) stated that in yoghurt produced from soy-cow milk mixture, pH values decreased more as the cow milk ratio increased in the mixture. The reason for the slower decrease in pH values of yoghurt with soy drink is that the starter cultures used to metabolize lactose faster than soy oligosaccharides (Cruz et al. 2009).

The amounts of titratable acidity of yoghurt samples in terms of lactic acid are given in Figure 2. There was no significant difference in titration acidity values between samples except the first day ($p>0.05$). During storage, the titration acidity of the samples was statistically significant except for the PY2 sample ($p<0.05$). Research shows that this is due to the β-galactosidase enzyme produced by yoghurt starter cultures (Shori 2013). This naturally increases the titration acidity while lowering the pH values.

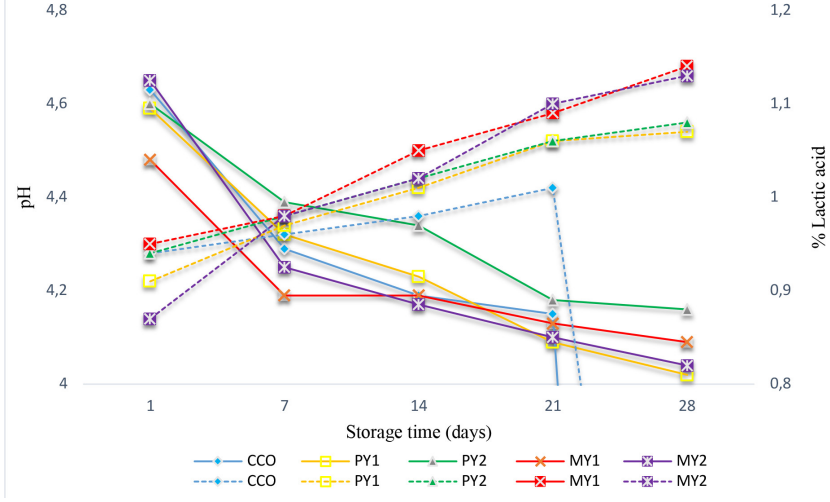

Figure 2- The pH values of the samples during storage (straight line); Changes in titratable acidity of samples (dashed line); CC0: control sample; PY1 and PY2: supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively

The syneresis values of yoghurt samples are given in Figure 3. Syneresis values between samples and during storage were statistically significant ($p<0.05$). In yoghurt samples, syneresis increased up to the 14th day, but at the end of the 28th day, small decreases were detected. It was determined that syneresis values increased with an increasing amount of extract. Tseng and Zhao (2013) stated that

the syneresis values of yoghurt enriched with grape pulp increased during storage. However, it is stated that the amount of syneresis increases with increasing grape pulp amount. Ingredients and amounts of ingredients to be used in yoghurt are important as they reorganize the protein gel matrix. During storage, pH values were reduced more slowly in extract-added samples than the control sample. The tightening of the gel structure of yoghurt and the decrease in syneresis were related to pH values (McCann et al. 2011). This expression is consistent with the slow decrease of pH values in the extract added samples until the 14th day and consequently increase of syneresis values.

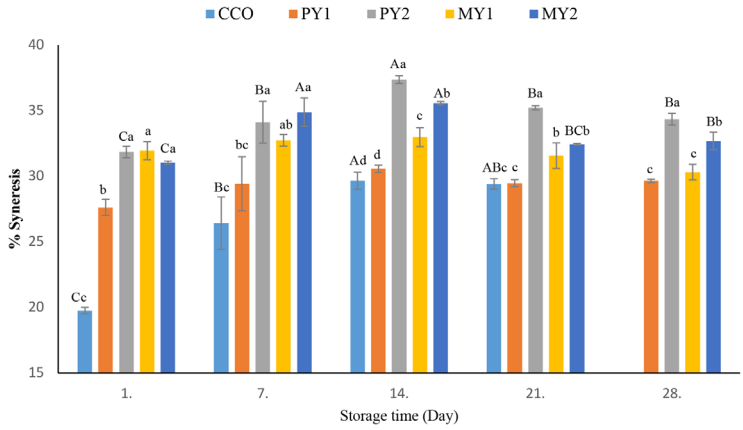

Figure 3- % syneresis values during storage of samples; CC0: control sample; PY1 and PY2: supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: Supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. Capital letters show the significant difference ($p<0.05$) between storage times of same treatment at some storage time. Small letters show the significant difference ($p<0.05$) between treatments. Values are means ± standard deviation of three replicates

3.5. Rheological properties of samples

The apparent viscosity of all the samples decreased as the shear rate increased, indicating a shear-thinning fluid behaviour (Figure 4). The viscosity of the control sample decreased continuously with increasing shear rate throughout the whole shear rate range studied. However, extract addition tended to alter the shape of viscosity curves. The addition of the extract caused a decrease in the apparent viscosity values of the samples. The rheological properties reflect the mouthfeel of yoghurts and are important because they significantly affect consumer preferences. The apparent viscosity values of yoghurt samples (η_{50}) are given in Figure 5. In all samples, η_{50} values of yoghurts increased during storage. The apparent viscosity values of the control and PY1 samples during storage were found to be statistically similar. All yoghurts exhibited characteristics of a weak viscoelastic gel with G'>G" at all frequencies investigated (Figure 6). G' is associated with the energy stored before deformation during the frequency sweep test and is related to the hardness of the gel structure network (Ferragut et al. 2009). G' values of all samples were always greater than G" values. Due to this situation the yoghurt system exhibited a solid-like behaviour. G' value is associated with the number and strength of the links between casein distributions in yoghurt (Sendra et al. 2010). G' values increased on the first day with the addition of 0.5% extract and microcapsule.

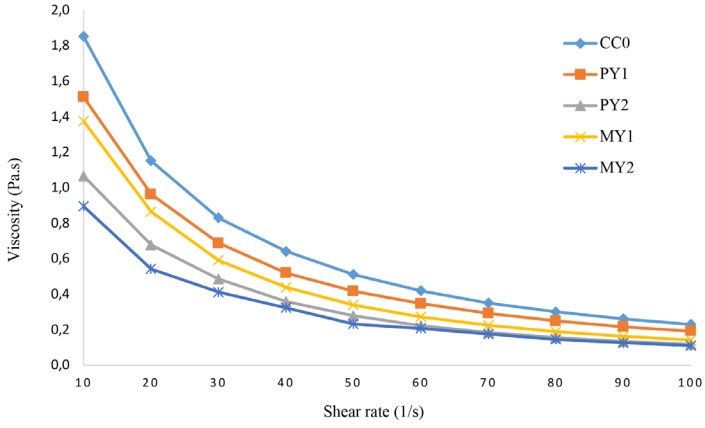

Figure 4- Apparent viscosity change due to shear rate in the 1st day samples. CC0: control sample; PY1 and PY2: yogurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: Yogurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively

tanδ (loss tangen) is a result of storage and loss modulus values (G"/G') and can provide more information about the viscoelastic properties of the samples (Figure 7). Low tanδ values mean stronger elastic behaviour of samples. Wang et al. (2020) stated that samples with low tanδ values can be perceived as more mouthful than higher ones. During storage, tanδ values decreased in all samples and are matched by solid-phase behaviour in this flow (Sendra et al. 2010).

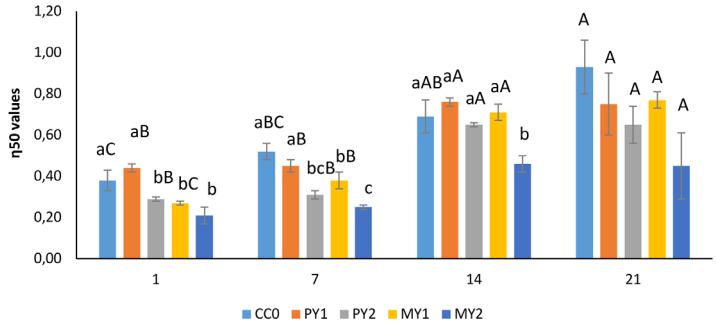

Figure 5- The apparent viscosity values of yogurt samples during storage (Pa.s). CC0: control sample; PY1 and PY2: yogurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: Yogurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. Capital letters show the significant difference (p<0.05) between storage times of same treatment at some storage time. Small letters show the significant difference (p<0.05) between treatments

The addition of extract in yoghurt caused rising in G' values compared to the control. A similar result was reported by Pan et al. (2019). On the 14th day, there was an increase in G' values and a decrease in tanδ values in all samples. This shows that yoghurts exhibit solid behaviour depending on storage.

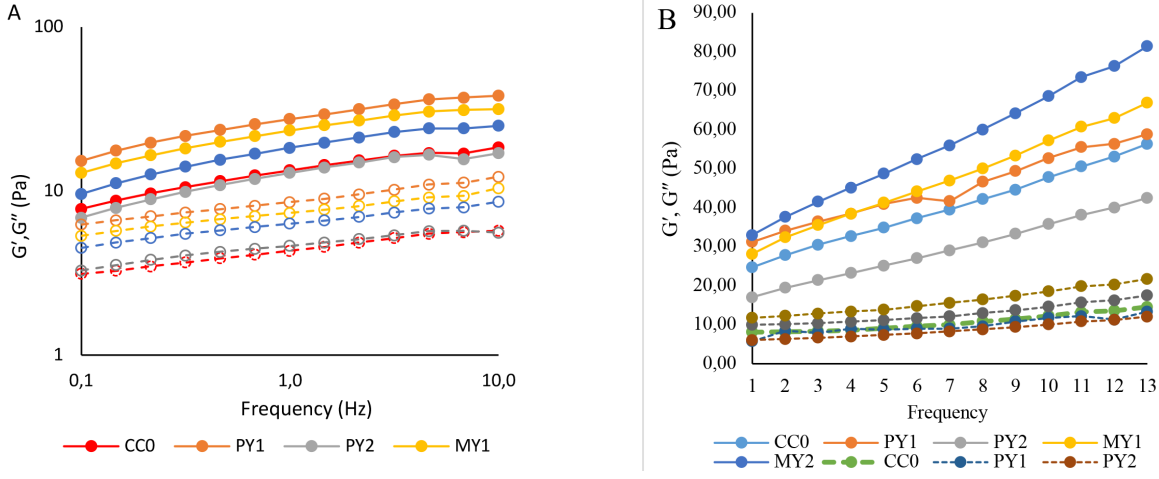

Figure 6- Frequency sweep curves of samples stored for 1 day (A), 14 days (B). CC0: control sample; PY1 and PY2: yogurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: yogurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. G', storage modulus, Pa (straight line) G", loss modulus, Pa (dashed line). G' and G" were obtained from 0.1 to 10 Hz of frequency sweep at 4 °C

3.6. Microbiological analyses

The average initial microbial counts on yoghurt samples were ~10^9 cfu/g. Counts of lactobacilli and streptococci were given in Table 2. Counts of lactobacilli and streptococci were significantly higher ($p<0.05$) in the control samples. However, it was found that the durability of PPE added yoghurts during storage was higher. There were statistically significant differences in lactobacilli numbers between samples and storage time ($p<0.05$). In the yoghurt supplemented with MPE, the number of lactobacilli was lower in all analysis days. The antimicrobial properties of PPE slowed the growth of lactobacilli but did not prevent the growth of the dominant flora. Research has shown that microencapsulated phenolic compounds retain their antiradical and antimicrobial properties for a longer time (Kaderides et al. 2015). In our study, the viability of lactobacilli in MPE added yoghurts was relatively low compared to other samples. At the end of 28 days of storage, lactic streptococci and streptococci numbers were decreased in all samples.

The added extract adversely affected the viability of Streptococci. Streptococci viability was less in MPE added yoghurts than PPE added yoghurts. As previously mentioned, microcapsules retain their antibacterial properties for a longer period and consequently limit microbial growth. Similar results were obtained by Bedani et al. (2014b). Alexandre et al. (2019) stated that fruit extracts are rich in phenolic compounds which are known as antimicrobial agents, inhibiting the growth of pathogenic bacteria and fungi. However, polyphenol-rich PPE exhibited low inhibitory activity against Lactobacillus and Streptococci strains.

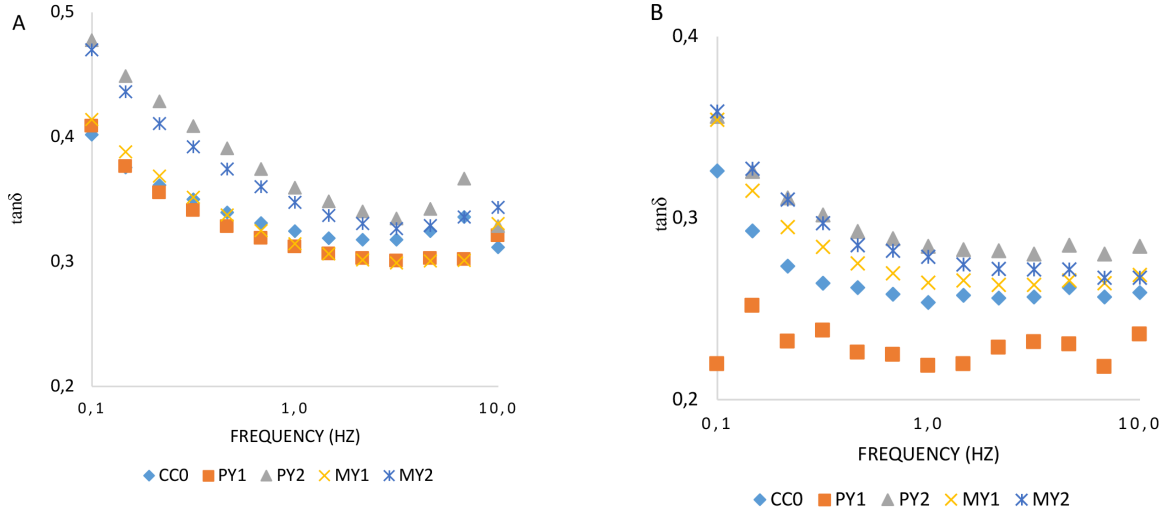

Figure 7- Loss tangent (tanδ) curves of samples stored for 1 day (A), 14 days (B). CC0: control sample; PY1 and PY2: yogurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: yogurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. tanδ were obtained from 0.1 to 10 Hz of frequency sweep at 4 °C

Any yeast-mould growth was observed in any yoghurt sample during 21 days of storage. On the 28th day of the storage period, only 2.6 log CFU/g of yeast and mould was observed in the control sample. On the 28th day, yeast and mould formation did not occur in PPE and MPE added yoghurts. These results show that PPE and MPE additive prevents yeast and mould growth in yoghurts and prolongs the shelf life of yoghurts (results not shown).

Table 2- Changes in microbial counts of yoghurt samples during the storage time (log cfu/mL)

		Days				
		1	***7***	***14***	***21***	***28***
Lactobacilli	**CC0**	9.08 ± 0.16^{Aa}	9.07 ± 0.06^{Aa}	8.53 ± 0.07^{bB}	$8.16\pm0.08C^{a}$	-
	PY1	8.87 ± 0.07^{abA}	8.85 ± 0.04^{Aa}	8.68 ± 0.01^{aB}	8.29 ± 0.09^{Ca}	7.63 ± 0.02^{Da}
	PY2	8.55 ± 0.20^{Abc}	8.76 ± 0.01^{Abc}	8.55 ± 0.01^{Ab}	8.21 ± 0.03^{Ba}	7.50 ± 0.00^{Cb}
	MY1	8.33 ± 0.11^{Ac}	8.44 ± 0.21^{Ab}	8.33 ± 0.04^{Ac}	7.93 ± 0.03^{Bb}	6.92 ± 0.06^{Cc}
	MY2	8.26 ± 0.15^{Ac}	8.40 ± 0.22^{Ab}	8.00 ± 0.01^{Bd}	7.65 ± 0.03^{Cc}	6.61 ± 0.18^{Dd}
Streptococci	**CC0**	9.23 ± 0.16^{Aa}	9.19 ± 0.05^{A}	9.24 ± 0.04^{Aa}	8.07 ± 0.01^{Ba}	-
	PY1	8.99 ± 0.08^{Ab}	8.95 ± 0.12^{A}	8.94 ± 0.01^{Ab}	7.87 ± 0.01^{Bb}	7.63 ± 0.00^{Ca}
	PY2	8.81 ± 0.01^{bc}	8.17 ± 0.72	8.88 ± 0.02^{b}	7.58 ± 0.06^{c}	6.88 ± 0.15^{bc}
	MY1	8.73 ± 0.00^{Ac}	8.88 ± 0.14^{A}	8.98 ± 0.11^{Ab}	7.86 ± 0.09^{Bb}	7.32 ± 0.12^{Cab}
	MY2	8.79 ± 0.06^{Abc}	8.55 ± 0.22^{A}	8.55 ± 0.14^{Ac}	7.26 ± 0.04^{Bd}	6.59 ± 0.28^{Cc}
Yeasts/mould	**CC0**	nd*	nd	nd	nd	2.60
	PY1	nd	nd	nd	nd	nd
	PY2	nd	nd	nd	nd	nd
	MY1	nd	nd	nd	nd	nd
	MY2	nd	nd	nd	nd	nd

CC0: Control sample, nd: Not determined, PY1 and PY2: Yoghurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: Yoghurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. Capital letters show the significant difference ($p<0.05$) between storage times. Small letters show the significant difference ($p<0.05$) between treatments. Values are means ± standard deviation of three replicates

3.7. Sensory evaluation

Changes in sensory properties of samples during storage were given in Table 3. The highest score was determined as 4.80 at control samples on the 7th day of storage while the lowest color and appearance score was determined as 3.00 at MY1 on the 1st day of storage. Although the color and appearance scores of the extract added yoghurts were higher than the 3 value, they were lower than the control samples. The differences between PPE and MPE added yoghurts were generally similar in terms of color and appearance scores. Similar situations were determined for texture scores of yoghurt. In the "taste and aroma" category, the sample that reached the highest score with 7.50 points was PY1 on the 28th day of storage. The sample with the lowest score was the control sample with a score of 5.58 on the 21st day of storage. The overall acceptability scores were detected between 5.57 and 7.38. For CCO sample was determined on the 21st day of storage while the lowest score for PE added yoghurts was determined at the MY2 sample on the 28th day of storage. The overall acceptability scores of the yoghurt decreased with PE adding but for PY1 and MY1 samples these scores increased during the storage time and the higher scores were determined on the 28th day of storage. Due to lack of habit to use soy products by panelists, the researchers suggested that the information given before the sensory tests of soy products would contribute to a more accurate assessment of the scores of the products (Bedani et al. 2014a; Drake & Gerard 2003).

Table 3- Changes in sensory properties of yoghurt samples during the storage time

	Days					
		1	***7***	***14***	***21***	***28***
Color and appearance	CC0	4.60 ± 0.14^{Aa}	4.80 ± 0.00^{A}	4.13 ± 0.00^{Ba}	4.50 ± 0.24^{ABa}	-
	PY1	3.70 ± 0.28^{Db}	3.90 ± 0.71^{C}	4.06 ± 0.08^{Ba}	4.09 ± 0.12^{Aab}	4.07 ± 0.50^{Ba}
	PY2	3.75 ± 0.35^{Ab}	3.38 ± 0.25^{E}	3.57 ± 0.09^{Db}	3.59 ± 0.12^{Cbc}	3.71 ± 0.01^{Bb}
	MY1	3.00 ± 0.14^{Ec}	3.82 ± 0.55^{B}	3.13 ± 0.18^{Dc}	3.89 ± 0.40^{Ab}	3.45 ± 0.05^{Cc}
	MY2	3.35 ± 0.21^{Abc}	3.35 ± 0.21^{A}	3.25 ± 0.00^{Bc}	3.25 ± 0.11^{Bc}	3.20 ± 0.10^{Cd}
Texture	CC0	4.65 ± 0.07^{a}	4.60 ± 0.00^{a}	4.44 ± 0.27^{a}	4.59 ± 0.12^{a}	-
	PY1	4.25 ± 0.07^{Ab}	3.85 ± 0.35^{Bb}	3.63 ± 0.35^{Db}	3.50 ± 0.00^{Ebc}	3.71 ± 0.20^{Cc}
	PY2	3.90 ± 0.00^{Ac}	3.25 ± 0.07^{Cc}	3.00 ± 0.00^{Db}	3.25 ± 0.35^{Cc}	3.85 ± 0.20^{Bb}
	MY1	3.55 ± 0.21^{Ed}	4.00 ± 0.14^{Bb}	3.59 ± 0.42^{Db}	3.95 ± 0.35^{Cb}	4.15 ± 0.02^{Aa}
	MY2	3.00 ± 0.14^{De}	3.79 ± 0.13^{Ab}	3.38 ± 0.00^{Cb}	2.92 ± 0.12^{Ec}	3.65 ± 0.01^{Bd}
Taste and aroma	CC0	6.45 ± 0.21^{BC}	7.40 ± 0.00^{aA}	6.98 ± 0.50^{AB}	5.58 ± 0.35^{bC}	-
	PY1	6.60 ± 0.42^{D}	6.70 ± 0.14^{Cb}	6.56 ± 0.25^{E}	7.42 ± 0.57^{Ba}	7.50 ± 0.30^{Aa}
	PY2	7.05 ± 0.50^{B}	6.84 ± 0.06^{Cb}	6.44 ± 0.08^{E}	7.33 ± 0.00^{Aa}	6.74 ± 0.10^{Dd}
	MY1	6.70 ± 0.28^{D}	7.00 ± 0.00^{Bb}	6.57 ± 0.81^{E}	7.17 ± 0.47^{Aa}	6.90 ± 0.33^{Cb}
	MY2	5.70 ± 0.28^{E}	7.45 ± 0.21^{Aa}	6.31 ± 0.03^{C}	6.84 ± 0.23^{Ba}	6.14 ± 0.20^{Dc}
Overall acceptability	CC0	7.30 ± 0.14^{Aa}	6.95 ± 0.00^{A}	7.38 ± 0.35^{Aa}	5.92 ± 0.35^{B}	-
	PY1	6.70 ± 0.57^{Cab}	6.65 ± 0.00^{D}	6.69 ± 0.08^{Cb}	6.84 ± 0.47^{B}	7.36 ± 0.01^{Aa}
	PY2	6.55 ± 0.07^{Bab}	6.40 ± 0.00^{D}	5.94 ± 0.08^{Ec}	6.58 ± 0.35^{A}	6.50 ± 0.01^{Cc}
	MY1	6.40 ± 0.28^{Db}	7.00 ± 0.42^{B}	6.07 ± 0.26^{Ec}	6.75 ± 0.35^{C}	7.31 ± 0.02^{Ab}
	MY2	5.95 ± 0.07^{Bb}	6.65 ± 0.50^{A}	5.75 ± 0.00^{Cc}	5.75 ± 0.11^{C}	5.57 ± 0.02^{Dd}

CC0: Control sample; PY1 and PY2: Yoghurts supplement with 0.5% and 1% pomegranate peel extract, respectively; MY1 and MY2: Yoghurts supplement with 0.5% and 1% microencapsulated pomegranate peel extract, respectively. Capital letters show the significant difference ($p<0.05$) between storage. Small letters show the significant difference ($p<0.05$) between treatments. Values are means ± standard deviation of three replicates

4. Conclusion

The addition of the extract also negatively affected the syneresis and apparent viscosity values. The addition of the extract increased the shelf life of the products by preventing the formation of yeast and mold in yoghurt samples. The yeast-mold formation did not occur in samples with a 1% extract addition even after 28 days. The addition of PPE and MPE was provided with reduced sensory scores due to its distinctive sour flavor when compared to the control. However, while the addition of PPE and MPE provided better preservation of sensory properties during storage, it caused weaknesses in the textural properties of yoghurts. This situation was also expressed by the panelists in their sensory analysis. In addition, it shows that they can be used as an antioxidant activity increaser in yogurt production

when the antioxidant values of PPE and MPE are examined. This is a promising conclusion that if the structure and textural properties can be improved, the use of PPE and MPE additives in yoghurts will become possible.

Data availability: Data are available on request due to privacy or other restrictions.

Authorship Contributions: Concept: H.T., E.B.E., Design: H.T., E.B.E., Data Collection or Processing: H.T., E.B.E., Analysis or Interpretation: H.T., E.B.E., Literature Search: H.T., E.B.E., Writing: H.T., E.B.E.

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