Rheology and sensory properties of microencapsulated propolis-enriched stirred-type yogurt

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Abstract

In this study, the rheological, sensory and synergetic properties of yogurt enriched with 0.5%, 1% and 2% microencapsulated propolis (MP) was investigated. The viscosity measurements of stir-type yoghurt were performed with Brookfield DV-II viscometer. Shear rate varying between 0.02 s⁻¹ and 100 s⁻¹ flow models was obtained for rising and descending curves with the Rheocalc v3.3 software. Three flow models—Power Law, Herschel–Bulkley, and Casson models—were evaluated. All samples used in this analysis showed non-Newtonian and pseudoplastic properties. During the 21-day storage period, the Power Law flow model was optimal.

Keywords: propolis, rheology, sensory, syneresis, yogurt

Introduction

Yogurt has been always in great demand because of the consumer’s search for a healthier diet because it is a noted functional food rich in high-quality protein content, folic acid, vitamins A and B, and calcium (Mathias et al., 2011). Yogurt is a coagulated milk product produced by lactic acid fermentation of Lactobacillus delbrueckii, subsp. bulgaricus and Streptococcus thermophilus. Yogurt has two major types: set yogurt and stirred yogurt (Anonymous, 2009).

Recently, the dairy industry has been closely related with the inclusion of nutrients in dairy products because of their beneficial effects on human health. Globally, consumption of yogurt has increased dramatically because of the research being done to make yogurt a more nutritious and consumable product (Aportela-Palacios et al., 2005). Taste, quality, consistency and viscosity are some of the main factors that play a role in product quality and acceptance (Mathias et al., 2011).

Propolis is a special resinous blend with strong antiviral, antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory effects, and is collected from the cones of trees, leaves, young shoots, shells, and buds and various plant oils, pollens, resin and waxy materials. These substances are harmonized through metabolic secretions of honeybees (Apis mellifera L). The potential application of propolis in pharmaceutical and food industries as a natural antioxidant and antimicrobial is promising (Jafari et al., 2022; Tavares et al., 2022). However, the use of propolis in foods is limited because of its strong flavor and aroma as well as its limited solubility in water. Microencapsulation technique appears an effective solution to overcome this problem and increase durability. Spray-drying propolis encapsulation is an alternative for avoiding unpleasant sensory qualities, protecting bioactivity and broadening the dose via a water-soluble encapsulation matrix. Because bioactive compounds frequently have extremely poor solubilities and bioavailabilities because of their hydrophobic nature, spray-dried powder is particularly important because it improves flavonoid
component solubility and absorption while minimizing heat degradation. Maltodextrin is a water-soluble matrix, often employed as a spray-drying agent because of its high solubility and strong bioactive ingredient retention. This feature has been linked to its proportion of dehydration, which results in the quick creation of thick skin and effective protection of the core component against oxygen transfer and potential degradation. Addition of additional component, such as various gums, can alter the physicochemical characteristics of powders as well as the preservation of bioactive chemicals (Baysan et al., 2019, 2021; Bostancı, 2017; Busch et al., 2017). The stability of microencapsulated propolis (MP) particles can last from 60 to 180 days according to the used encapsulation wall material and encapsulation process conditions (Sa et al., 2023; Silva et al., 2013). On the other hand, limited information is available on the production of yogurt, which incorporates powdered microcapsules of propolis extract.

Yoghurt has shear-thinning, thixotropic, viscoelastic and tensile rheological properties because of the internal microstructure, which forms a loose cross-linked gel structure due to interaction between milk proteins and fat molecules. In addition to viscosity properties, sensory properties, appearance and odor are important factors in quality and general consumer acceptance. In this context, understanding the mechanisms involved in texture formation in yoghurt and the effect of processing conditions on texture development can help improve yoghurt quality (Lee and Lucey, 2010; Mullineux and Simmons, 2008).

Ramaswamy and Basak (1992) investigated the effect of pectin and raspberry concentrate on the rheological behavior of stirred yogurt; the researchers incorporated pectin and raspberry concentrate in commercially stirred yogurt samples for increasing its consistency. Yogurt with pectin was more shear stable, compared to yogurt with raspberry concentrate.

Aportela-Palacios et al. (2005) manufactured a semi-stirred yogurt fortified with different levels of fibers and calcium salt, and analyzed the influence of the added ingredients on flow and physicochemical properties. It was determined that with increasing fiber (1.5%, 3.0% and 4.5% by weight), acidity of the samples increased with decrease in syneresis. In the cited study, natural bran had a greater effect on consistency than the toasted bran, and it was discovered that the yogurt flavored with pina colada had higher viscosity than yogurt flavored with pineapple.

In the study, sour cherry pulp was added to yoghurt (in proportion of 0%, 8%, 12% and 16%) and its effect on physicochemical properties, phenolic content, antioxidant activity and sensory properties were investigated. The data were collected through 14 days of cold storage. The increasing concentration of sour cherry pulp in yoghurt resulted in increase of pH and whey separation, whereas decrease in the values of other parameters, such as total solid, fat, protein, ash, titratable acidity and viscosity. It was reported that yoghurts produced with sour cherry are rich in phenolics and have a strong antioxidant activity. Thus, the utilization of sour cherry in the production of yoghurt was recommended (Şengül et al., 2012).

Dönmez et al. (2017) suggested that the effect of green tea extract on the proportion of syneresis was concentration-dependent in set yogurts. The extract decreased proportion of syneresis if it was added at low levels (0.02%), but it increased syneresis if added at 2%. In the study, the Herschel–Bulkley model parameters indicated that the consistency of the control was considerably lower than that of green coffee powder yogurts during 14 days of storage, whereas it was higher at the end of storage period. Since both green tea powder and green coffee powder have different profiles and concentrations of polyphenols, they behaved differently in acidified gel networks of set yogurt, modifying its rheological behavior.

In another study (Baba et al., 2018), authors observed the quality characteristics of yoghurt fortified with guar gum, flaxseed and walnut oil at different proportions; it was revealed that the increasing concentrations improved the quality properties of yoghurt. Guar gum affected yoghurt rheology in a concentration-dependent manner with best results at 0.025%. In addition, walnut oil was a better option for the supplementation of yogurt than flaxseed oil, because walnut oil showed improved Ω-6–Ω-3 ratio (Ω-6:Ω-3), oxidative stability and antioxidant activity. Based on these results, manufactures can improve the oil formulations used for producing fortified yogurt.

Ünal et al. (2003) investigated the effects of locust bean gum (LBG), dry matter concentrations and storage time on the physical properties of low fat set yoghurt. After 1, 7 and 14 days of processing at 4°C, viscosity, water-holding capacity (WHC), syneresis, pH and acidity analyses were applied to the samples. In this study, viscosity increased with increasing dry solid content.

Yadav et al. (2018) evaluated the stability of grape seed extract (GSE) microencapsules in yoghurt and their effect on its antioxidant and physicochemical properties during preparation and storage. The effect of encapsulated (En) and nonencapsulated (NE) GSE at 1% level added to the milk before its fermentation was studied, and physicochemical properties, total phenolic content and antioxidant activity of the obtained yoghurt were...
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Materials and Methods

Materials

Propolis was obtained from a local farm facilitating in Sivas province. Starter culture YC-X16 (thermophilic, freeze-dried lactic culture YoFlex) and pasteurized milk (Şütas, 3% protein and 3% fat) were obtained from the market.

Preparation of propolis extract and microencapsulation

Approximately 14 g of propolis were dissolved in 100 mL of ethanol (Sigma-Aldrich, Germany) with stirring for 24 h. It was then filtered under vacuum with a Buechner funnel using filter paper. To remove all remaining wax, the ethanolic extract was kept in a freezer (-20°C) for 10 h and centrifuged twice (2-16PK, Sigma-Aldrich) for 10 min at 4,500 rpm at 5°C. Clear liquid remaining in the supernatant was removed and stored at 4°C (Busch et al., 2017).

For microencapsulation process, laboratory-scale Buchi B-290 (Flawil, Switzerland) mini-spray drier was used at TÜBİTAK MAM (İstanbul, Turkey). The feed flow rate was 4 mL/min, the air inlet temperature was 120°C, and the outlet temperature varied between 65°C and 70°C. Microencapsulation was performed for 8 h and the obtained MP extract was stored at 4°C in a refrigerator (Bostancı, 2017; Busch et al., 2017).

Preparation of MP-fortified yogurt

To produce stirred-type yoghurt product, pasteurized milk was first heated to 42°C and YC-X16 yogurt culture (3 g/100 mL) was added swiftly. The samples were allowed to incubate at 42°C for 8 h to obtain pH 4.6. Following incubation, varying amounts of MP were added (0%, 0.5%, 1% and 2% w/w) to yoghurt samples. All the samples were stirred and stored at 4°C in 250-mL plastic closed lid containers. The analyses were carried out on 0th, 7th, 14th and 21st days.

pH analysis

The pH values were monitored by Sentix 41 electrode hand-type WTW 315i Set (Weilheim, Germany) pH meter at room temperature.

Rheological analysis

Instrumental rheological measures were determined with a Brookfield Viscometer (DV-II; Brookfield Engineering Laboratories, Middleboro, MA, US) using a
programmable analysis process with yogurt at 20°C. The yogurt samples were gently stirred 10 times for homogenization. Then, approximately 400 mL of the sample was transferred into a beaker placed in a water bath and the temperature of the water bath was set to 20 ± 1°C to prevent temperature fluctuations during measurements. A set of velocities or an upward curve was programmed from 0 to 100 rpm and the corresponding torque values were registered. From pairs of rotational velocity-torque values and applying the manufacturer-given constants for the V75b spindle, the apparent shear and shear stress values were represented graphically and evaluated mathematically. The corresponding shear stress (τ) data were computed by a Rheocalc v3.3 software (Brookfield). In order to characterize non-Newtonian food fluids, Power Law, Herschel–Bulkley, and Casson models were used normally. From the flow curves, the rheological parameters were evaluated by application of the Power Law, Herschel–Bulkley, and Casson models (Equations 1–3).

To obtain flow and viscosity curves, analyze the variation of tension and viscosity, respectively, as a function of shear rate, which ranged from 0.02 s⁻¹ to 100 s⁻¹ (rising curve) and from 100 s⁻¹ to 0.02 s⁻¹ (descending curve), enabling the determination of hysteresis as the area between curves (Mathias et al., 2011):

\[ \tau = K \gamma^n, \]  
\[ \tau = \tau_0 + K \gamma^n, \]  
\[ \tau^{1/2} = \tau_0^{1/2} + (K \gamma^1)^{1/2}, \]  

where \( \tau \) is the shear stress (Pa), \( \tau_0 \) is the yield strength (Pa), \( K \) is the consistency coefficient (Pa.sⁿ), \( \gamma \) is the apparent shear rate (1/s), and \( n \) is the flow-behavior index (dimensionless) that describes the flow behavior of the fluid as shear-thinning/pseudoplastic \((n < 1)\) or shear-thickening/dilation \((n > 1)\) (Aportela-Palacios et al., 2005; Espírito-Santo et al., 2013). Apparent viscosity \( (\eta_{app}) \) was calculated as \( \gamma = 2 \text{ s}^{-1} \) in downward flow curves (Espírito-Santo et al., 2013).

**Syneresis analysis**

Yogurt samples, 25 g, were taken and filtered for 4 h through a filter paper. Then, the collected liquid was measured in a measuring flask.

**Sensory analysis**

The experimental yogurt samples coded with random numbers were placed on white plates and presented together to panel members in daylight. Panel members were asked to evaluate each sample in turn, covering a list of judged parameter attributes using a hedonic scale as “1” being the worst and “10” being the best. The scores given by panelists to each sample were noted separately.

The consistency of the samples was judged by gentle mixing of yogurt with a spoon and by tasting. To determine consistency by tasting, a spoonful of yogurt is taken and spread on tongue. The yogurts were evaluated on the 1st, 7th, 14th and 21st day of storing by eight untrained panelists familiar with yogurt (five females and 3 males, age ranging from 20 to 55 years). Fresh water was provided for mouth neutralization between tasting of samples.

**Statistical evaluation**

Statistical Package for Social Sciences (SPSS) 22 was used for statistical analysis of the data. One-way ANOVA test was used to determine the mean scores of more than two groups, and Tukey’s post-hoc test was used to determine differences between homogeneously distributed groups \((p < 0.05)\). In the experiments, three replicates were performed and the mean values were calculated.

**Results and Discussion**

**pH analysis**

During 21 days of storage, the pH values of the samples decreased and this decrease was statistically significant \((p < 0.05; \text{Table 1})\). The highest pH values were observed on the ﬁrst day of storage, and consequently pH decreased, resulting in increased acidity during the following days. The varying MP ratios also caused signiﬁcant differences in pH values (Table 1). This decrease helped the inhibition of pathogenic bacteria. Reduced pH and increased acidity are typical in refrigerated yogurts because of the metabolism of *L. acidophilus*, which triggered the development of lactic acid from the fermentation of lactose, considered a homofermentative bacteria (Batista et al., 2015). This attribute of acidophilic-fermented milk encourages the inhibition of undesired microorganism growth, such as that of degrading pathogenic bacteria. Because excessive repulsion of charge affects syneresis, pH regulation in yogurt is extremely important (Molina et al., 2019). Furthermore, one of the most signiﬁcant characteristics of ﬂavor in yogurt is acidity, and the ideal pH of yogurt is often close to 4.4. Some customers desire less acidic yogurt, which is considered as “less tasty” (Batista et al., 2015).

**Rheological analysis**

The semisolid texture of the set yoghurt gel is a product of 3D network of milk proteins being formed. Owing
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Table 1. pH values of yogurt samples.

<table>
<thead>
<tr>
<th>MP content</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.31 ± 0.00aA</td>
<td>4.16 ± 0.00aA</td>
<td>4.10 ± 0.00aA</td>
<td>4.09 ± 0.00aA</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.32 ± 0.00aA</td>
<td>4.25 ± 0.00aA</td>
<td>4.22 ± 0.00aA</td>
<td>4.18 ± 0.00aA</td>
</tr>
<tr>
<td>1%</td>
<td>4.32 ± 0.00aA</td>
<td>4.25 ± 0.00aA</td>
<td>4.22 ± 0.00aA</td>
<td>4.18 ± 0.00aA</td>
</tr>
<tr>
<td>2%</td>
<td>4.39 ± 0.00aA</td>
<td>4.24 ± 0.00aA</td>
<td>4.17 ± 0.00aA</td>
<td>4.14 ± 0.00aA</td>
</tr>
</tbody>
</table>

MP: microencapsulated propolis.

a,b,c,d letters are significant differences between rows at \( p < 0.05 \).

A,B,C,D letters are significant differences between columns at \( p < 0.05 \).

to the release of acids because of microbial activity, the main factor responsible for milk gelation is reduction in net negative charge of casein micelles. During fermentation, casein micelles, together with denatured whey proteins, aggregate through hydrophobic and electrostatic bonds into chains and clusters, which regulate the structure of yoghurt. The aggregation of casein micelles begins at an approximate pH of 5.3, which also induces colloidal calcium phosphate solubilization. Further reduction of pH to below 5.0 causes a more complex and extensive interconnection of casein micelles, and the gel reaches its maximum firmness at pH 4.6—the casein isoelectric point (Paseephol et al., 2008).

All yogurt specimens displayed non-Newtonian and pseudoplastic fluid behavior, with decreased viscosity and increased shear speed, regardless of the volume or type of thickener used. This could be due to the physical disruption of weak bonds between the molecules of yoghurt and reduced interaction energy between them. Several studies stated yogurt as a pseudoplastic fluid (Gonçalvez et al., 2009; Horne, 1998; Lucey, 2002; Mathias et al., 2011; Paseephol et al., 2008). Table 2 shows the rheological parameters calculated using Rheocalc v3.3 software (Brookfield Engineering) for rheological models (Figure 1).

Apparently, the shear rate in upward flow curves fitted by Power Law model resulted in adequate correlation coefficient, \( R^2 \geq 0.971 \), indicating that this model is more adequate to represent the experimental data (Table 2). The mean values of flow-behavior index, \( n < 1 \), thus pointing out a pseudoplastic behavior of MP-added yoghurt samples. These results showed that the flow pattern, determined as non-Newtonian pseudoplastic in all yogurt samples, neglected the effect of different additives to yogurt. Dönmez et al. (2017) and Mathias et al. (2011) achieved similar results in their studies.

![Figure 1](image). Examples of the plots of rheological model fits for yogurts by Rheocalc v3.3 software at 20°C added with (A) Herschel–Bulkley model fit for 1% MP-added yoghurt on 21st day and (B) Casson model fit for 2% MP-added yoghurt on 14th day.
Table 2. Flow behavior of control and MP-added yoghurt samples predicted by Power Law, Herschel–Bulkley, and Casson models during the 21 days of cold storage.

<table>
<thead>
<tr>
<th>Days</th>
<th>Group name</th>
<th>Power Law model</th>
<th>Herschel–Bulkley model</th>
<th>Casson model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$K$</td>
<td>$n$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0.032</td>
<td>0.522</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>0.091</td>
<td>0.395</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0.014</td>
<td>0.463</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>0.041</td>
<td>0.379</td>
<td>0.979</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>1.928</td>
<td>0.202</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>1.747</td>
<td>0.242</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>1.950</td>
<td>0.290</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>2.308</td>
<td>0.363</td>
<td>0.975</td>
</tr>
<tr>
<td>14</td>
<td>Control</td>
<td>0.655</td>
<td>0.218</td>
<td>0.971</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>1.086</td>
<td>0.167</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0.634</td>
<td>0.209</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>0.314</td>
<td>0.269</td>
<td>0.979</td>
</tr>
<tr>
<td>21</td>
<td>Control</td>
<td>0.790</td>
<td>0.175</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>0.277</td>
<td>0.255</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0.400</td>
<td>0.220</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>0.821</td>
<td>0.157</td>
<td>0.987</td>
</tr>
</tbody>
</table>

MP: microencapsulated propolis.

The calculated apparent viscosity decreased with rise in shear rate (graph not shown). This is anticipated as related to the shear-thinning phenomenon, which also revealed the pseudoplastic behavior of samples (Espírito-Santo et al., 2013; Fischer et al., 2009). As seen in Figure 2, the calculated apparent viscosity decreases with storage time.

**Syneresis analysis**

Syneresis (released whey) is one of the most important physical parameters that affect the quality of milk products. The proportion of syneresis increased in all samples during storage, and this increase was statistically significant ($p < 0.05$) (Table 3). This phenomenon has been explained in literature by decrease in pH during storage, which has a contracting effect on casein micelle matrix that enforces more release of serum (Estrada et al., 2011; Ghorbanzade et al., 2017; Salvador and Fiszman, 2004; Santos et al., 2019; Staffolo et al., 2004). Syneresis is associated with the gel network's instability and its reduced ability to entrap all serum stages (Izadi et al., 2015). Addition of MP appeared to have no effect on the syneresis proportion of samples (Table 3). Different quantities of propolis induced variances in the syneresis levels of samples but the findings were not statistically significant ($p < 0.05$) (Aportela-Palacios et al., 2005; Ghorbanzade et al., 2017; Tosun et al., 2011).

**Sensory analysis**

The mean sensory scores provided by all panelists are shown in Table 4 ($p < 0.05$). A statistically significant difference was discovered in consistency between the control and 2% MP addition in yoghurt samples on 7th day. Apart from this result, storage has no significant effect on sensory testing.

**Conclusions**

Microencapsulated propolis extract could be a suitable functional ingredient for production of yoghurt. Enriching yoghurt with MP extract loosened yoghurt's structure and decreased apparent viscosity during storage. All yoghurt samples showed non-Newtonian pseudoplastic behavior. Power Law model represented the flow curves of yoghurt samples more accurately. The flow-behavior index ($n$) < 1 indicates the pseudoplastic character of samples. Microencapsulated propolis extract is a promising ingredient from a technological point of view. In addition, these natural ingredients draw attention due to their health-promoting properties, which could be easily adopted by consumers.
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Figure 2. Apparent viscosity ($\eta_{app}$) calculated at $\gamma' = 2$ s$^{-1}$.

Table 3. Physicochemical properties of MP-fortified yogurt (n = 2).

<table>
<thead>
<tr>
<th>MP content</th>
<th>Days</th>
<th>Syneresis analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>28.04 ± 0.06$^{aA}$</td>
<td>29.78 ± 0.02$^{aA}$</td>
</tr>
<tr>
<td>0.5%</td>
<td>24.97 ± 0.00$^{aA}$</td>
<td>30.28 ± 0.00$^{aB}$</td>
</tr>
<tr>
<td>1%</td>
<td>29.26 ± 0.00$^{aA}$</td>
<td>30.29 ± 0.00$^{aA}$</td>
</tr>
<tr>
<td>2%</td>
<td>26.29 ± 0.00$^{aA}$</td>
<td>40.05 ± 0.00$^{bB}$</td>
</tr>
</tbody>
</table>

MP: microencapsulated propolis.

$^{a,b,c,d}$ letters are significant differences between columns at $p < 0.05$.

$^{A,B,C,D}$ letters are significant differences between rows at $p < 0.05$.

Table 4. Results of sensory test on consistency.

<table>
<thead>
<tr>
<th>MP content</th>
<th>Days</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>7.50 ± 2.05$^{aA}$</td>
<td>8.00 ± 1.41$^{aB}$</td>
</tr>
<tr>
<td>0.5%</td>
<td>5.63 ± 1.77$^{aA}$</td>
<td>6.75 ± 2.12$^{aB}$</td>
</tr>
<tr>
<td>1%</td>
<td>6.13 ± 1.95$^{aA}$</td>
<td>5.50 ± 1.92$^{aB}$</td>
</tr>
<tr>
<td>2%</td>
<td>5.25 ± 1.83$^{aA}$</td>
<td>6.50 ± 1.51$^{aB}$</td>
</tr>
</tbody>
</table>

MP: microencapsulated propolis.

$^{a,b,c,d}$ letters are significant differences between rows at $p < 0.05$.

$^{A,B,C,D}$ letters are significant differences between columns at $p < 0.05$.

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