ABSTRACT

Adding functional ingredients is an important method to develop functional dairy products. Mulberry pomace (MPo), a byproduct of mulberry fruit processing, is rich in phenolic compounds and anthocyanins and can be served as the functional ingredient in functional dairy products. The aim of this work was to prepare a functional flavored yogurt by incorporating MPo into stirred yogurt and to investigate the effects of MPo on the physicochemical and textural properties of the product during cold storage. We supplemented MPo powder up to 3% (wt/wt) in fermented milk, and the changes in color, pH, titratable acidity (TA), total phenol content (TPC), total anthocyanin content (TAC), water-holding capacity, rheological behavior, texture, and microstructure of the functional flavored yogurt were monitored during storage under 4°C for 28 d. The MPo powder brought a pink to dark red color to the yogurt, decreased the lightness ($L^*$) and yellow-blue color ($b^*$) values, increased the red-green color ($a^*$) values, decreased the pH value, and increased the contents of TA, TPC, and TAC in a dose-dependent manner. The addition of MPo at 1%, 2%, and 3% (wt/wt) significantly increased water-holding capacity, consistency, viscosity, and viscosity index, and reduced firmness of yogurt samples. Supplementation of MPo significantly reduced the pore spaces and channels inside the samples and improved microstructure of the functional yogurt. During the 28 d of cold storage, MPo-fortified yogurt samples kept relatively constant color, although their $L^*$, $a^*$, and $b^*$ showed a decreasing tendency. The pH of all yogurt samples gradually decreased with increasing of TA. Interestingly, TPC and TAC contents and the texture parameters of MPo-fortified yogurt increased gradually and continuously during the 28 d of cold storage. Mulberry pomace is beneficial to improve the physicochemical and textural properties of yogurt and has the potential as a natural stabilizer to be used in functional yogurt rich in phytochemicals.

Key words: mulberry pomace, stirred yogurt, rheology, phenolic compound

INTRODUCTION

Increasing health awareness of consumers promotes the development of functional foods. On the one hand, with the improvement of people’s living standards, the incidence of various chronic diseases such as hyperglycemia, hyperlipidemia, hypertension, tumor, and other diseases is gradually increasing, which seriously affects the health of middle-aged and elderly people (Halpin et al., 2010). On the other hand, with the outbreak of epidemics such as COVID-19, infectious diseases are still a major threat to human survival, and people find it necessary to improve their immunity to resist diseases (Marois et al., 2020). Medical costs and toxic and side effects of many drugs are the uppermost factors forcing people to find relatively cheap, effective, and safe ways to protect their health; therefore, consumers are paying increased attention to functional foods (Özer and Kirmaci, 2010).

Milk and dairy products are very important foods in the world. After decades of rapid development, the global dairy industry experienced a depressed product price and trade situation in 2014 to 2016 (Vitaliano, 2016). In recent years, the yield of dairy products in many countries exceeds their social demand, and the dairy industry is facing new challenges (Pouch and Trouvé, 2018). As consumers become more health conscious, their demands for functional dairy products are increasing rapidly. At present, functional dairy products occupy a significant space in the functional...
food market (Özer and Kirmaci, 2010). Functional dairy foods, enriched with functional food components originating from dairy and nondairy sources, demonstrate health benefits beyond their basic nutritional value (Özer and Kirmaci, 2010). They can regulate our physiological functions and prevent and treat diseases (Martins et al., 2018). Functional dairy foods can be divided into natural and additive types, according to their production methods. The former is produced by adjusting the feed formula of dairy cows to accumulate the functional components in milk (Xu et al., 2017), whereas the latter is developed by adding different functional ingredients to dairy products (Sukhikh et al., 2019). However, both the biological barrier of digestive tract and physiological regulation of dairy cows limit the types and contents of accumulated functional components in milk. Thus, adding exogenous functional components is an important method to produce functional dairy products.

Yogurt itself is a functional dairy product, as it contains lactic acid bacteria (LAB) and their metabolites. In addition, there are more probiotics and less allergens such as lactose in the product after lactic acid fermentation (Markowiak and Śliżewska, 2017). In recent years, plant materials rich in functional components have been supplemented to yogurt to strengthen functional activities of yogurt products. The reported additives include grape (de Souza Azevedo et al., 2018), pomegranate (Trigueros et al., 2014), blueberry (Felix da Silva et al., 2017), strawberry (Gunenc et al., 2015), roselle (Levya Daniel et al., 2013), sea-buckthorn (Gunenc et al., 2016), and apple pomace (Wang et al., 2019b, 2020), among others.

Mature mulberry fruit is rich in anthocyanins, non-anthocyanin phenolics, polysaccharides, and other functional components (Zhang et al., 2008; Wang et al., 2019a). Mulberry pomace (MPo), a byproduct of mulberry juice production and wine making, contains even higher contents of polyphenols (flavonoids and anthocyanins) and fiber than mulberry fruit (Kang et al., 2015; Khodaiyan and Parastouei, 2020). It could be a good ingredient for developing functional dairy foods. However, both the biological barrier of digestive tract and physiological regulation of dairy cows limit the types and contents of accumulated functional components in milk. Thus, adding exogenous functional components is an important method to produce functional dairy products.

**Preparation of MPo Powder**

After thawing, the frozen MPo was dried in a heat pump dryer (GHRH-20, Guangdong Hongke Agricultural Machinery R&D Co., Ltd.) at 70°C for 3 h and then at 35°C until the moisture content was less than 10%. Dried MPo was ground using a plant pulverizer (BJ-300A, Deing Baijie Electric Appliance Co., Ltd.) at 70°C for 3 h and then at 35°C until the moisture content was less than 10%. Dried MPo was ground using a plant pulverizer (BJ-300A, Deing Baijie Electric Appliance Co., Ltd.) at 70°C for 3 h and then at 35°C until the moisture content was less than 10%. Dried MPo was ground using a plant pulverizer (BJ-300A, Deing Baijie Electric Appliance Co., Ltd.) at 70°C for 3 h and then at 35°C until the moisture content was less than 10%. Dried MPo was ground using a plant pulverizer (BJ-300A, Deing Baijie Electric Appliance Co., Ltd.) at 70°C for 3 h and then at 35°C until the moisture content was less than 10%.

**Production of Stirred Flavored Yogurt**

Yogurt samples were prepared following the method of Yekta and Ansari (2019) with some modifications, and the production flow-chart is shown in Figure 1. Skim milk powder and water (1.0/9.5, wt/vol) were thoroughly mixed, pasteurized at 85°C for 30 min, and then cooled to 42°C. The cooled reconstituted milk was inoculated with 1 g/L of starter culture with viable LAB count of 9.01 ± 0.03 log_{10} cfu g^{-1}, fermented in an incubator at 42°C until the final pH reached 4.6, and then cooled to 10°C. The MPo-enhanced flavored yogurt was prepared by adding 1%, 2%, and 3% (wt/wt) MPo powder to the cooled plain yogurt, respectively. After stirring gently for 3 min, fortified flavored yogurt containing MPo was distributed into 150-mL sterilized glass containers and stored in a dark environment at

**MATERIALS AND METHODS**

**Materials**

The MPo, directly frozen after squeezing the juice from fresh mulberry fruit and stored under −18°C, was purchased from Guangdong Bosun Health Food Co., Ltd. The starter culture (classic type, containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, Angel Yeast Co., Ltd.), with viable count (± SD) of 9.01 ± 0.03 log_{10} cfu g^{-1}, and skim milk powder were purchased from a local supermarket. Folin-Ciocalteu reagent (Macklin) and the remaining reagents were of analytical grade or authentic standard chemicals.

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4°C. Samples were taken at 1, 7, 14, 21, and 28 d for quality evaluation.

**Color, pH, TA, and Probiotic Viability**

The color was measured using a colorimeter (UltraScan VIS, HunterLab). The yogurt samples (6 g) were packed in test tubes. The CIELab parameters lightness value ($L^*$), red-green value ($a^*$), and yellow-blue value ($b^*$), hue angle $[\tan^{-1} (b^*/a^*)]$, and chroma $[(a^{*2} + b^{*2})^{1/2}]$ were reported. The $L^*$ value ranges from 0 (black) to 100 (white). Coordinate $a^*$ represents red (positive) to green (negative); $b^*$ represents yellow (positive) to blue (negative). The pH value of the yogurt was measured using a digital pH meter (PB-10, Sartorius). Yogurt extract, prepared by diluting yogurt samples with distilled water (1:4), was used to determine the TA content according to GB 5009.239–2016 using an automatic titrator (ZDJ-4B, Lemag). It was titrated with 0.1 M NaOH to pH 8.1, and the TA was calculated. The results were expressed as the percentage of lactic acid. Probiotic viability was tested by serial dilution, pour-plating, and anaerobic incubation method (GB4789.35–2016).

**Total Phenol and Total Anthocyanin Content**

The preparations of yogurt samples were carried out according to Trigueros et al. (2014) with some modifications. We extracted 2.0-g samples of yogurt using 60 mL of 60% (vol/vol) acidified ethanol in water with ultrasonic treatment at 200 W for 2 h twice, followed by vacuum filtration. The 2 filtrates were combined, subjected to rotary evaporation at 50°C, and centrifuged at 3,570 × $g$ for 10 min at 4°C. The supernatants were diluted to 25 mL for measurement. The TPC was determined using the Folin-Ciocalteu method (Yadav et al., 2018) by measuring the absorbance at 760 nm using a spectrophotometer (UV-1800, Shimadzu). The results were expressed as milligram of GAE per gram of yogurt. The TAC was determined using the pH differential method (Liu et al., 2004) on a spectrophotometer (UV-1800, Shimadzu). The results were expressed as microgram of C3GE per gram of yogurt.

**Water-Holding Capacity**

Water-holding capacity (WHC) of yogurt was determined according to the method of Luana et al. (2014). Fifteen grams of sample were centrifuged for 10 min at 3,570 × $g$ at 25°C (TGL-16M, CENCE). The expelled water was removed and weighed. The percentage of WHC was defined according to the following equation: 

$$\text{WHC} = \left(\frac{\text{sample weight} - \text{expelled water}}{\text{sample weight}}\right) \times 100.$$

**Rheology Measurement**

Rheological test was carried out based on the method of Sun-Waterhouse et al. (2012) with some improvements. The rheological measurement was performed on an AR1500EX rheometer (TA Instruments Co., Ltd.) equipped with a high-performance drag cup, optical encoder, normal force sensor, 40-mm flat plate special fixture, and Peltier temperature control system.

The steady state viscosity of the stirred yogurt sample was measured at shear rates of 1 to 300 s$^{-1}$. Before the time sweep test of elastic modulus, a 100 s$^{-1}$ rotating shear rate was applied to all samples, and the yogurt structure recovery was obtained at 4°C for 2 h. The test was performed under the conditions of a constant strain of 0.5% and frequency of 1 Hz. After the recovery test, a frequency sweep test was performed with frequency ranging from 0.1 Hz to 100 Hz. During
the test, the storage modulus ($G'$), loss modulus ($G''$), and loss tangent (tan δ) were monitored.

**Texture Analysis**

The texture analysis was carried out according to the method of Wang et al. (2020) with some modifications. A backward extrusion test was performed in stirred yogurt using TA-XT Plus texture analyzer (Stable Micro Systems). The test parameters were as follows: cylindrical probe diameter (36 mm), test speed [1.0 mm/s (before), 1.0 mm/s (during), 2.0 mm/s (after)], test distance (20 mm), and surface triggering force (10 g). Stirred yogurt was transferred into a 125-mL glass container of 64 mm × 70 mm (diameter × height). The firmness (N), consistency (N × s; total positive area), cohesiveness (N; maximum adhesive force), and viscosity index (N × s; total negative area) were calculated using texture exponent software (Exponent, version 6.11.16.0, Stable Micro Systems).

**Confocal Laser Scanning Microscopy Analysis**

A confocal scanning inverted microscope (Zeiss LSM710, Zeiss) was used to observe the microstructure of the yogurt samples with a confocal filter block (Zeiss LSM710) and a 63× oil objective lens. A digital image resolution of 1,240 × 1,240 pixels was set in ZEN (version 2009, Zeiss LSM710).

Yogurt samples were prepared according to the method of Kristo et al. (2011). Milk protein was stained with 0.2% (wt/vol) rhodamine B aqueous solution (excitation wavelength 543 nm and emission wavelength 625 nm). Five milliliters of agitated yogurt sample was mixed with 20 μL of rhodamine B solution. The stained samples were transferred to a glass slide with a cavity and cover. The stained specimens were stored at 4°C for 1 h before confocal laser scanning microscopy analysis. The microstructure of the samples in multiple areas was observed, and the representative microscopic images were selected.

**Statistical Analysis**

All experiments were repeated 3 times. The results are expressed as mean ± standard deviation. SPSS 21 (IBM) was used for statistical analysis, and Origin 8.0 software (Origin Lab) was used for drawing. One-way ANOVA and Tukey tests were performed.

**RESULTS AND DISCUSSION**

**Physical and Chemical Properties of MPo-Fortified Flavored Yogurt**

Color is an important physical index for milk quality. We found that the product showed pink to dark red after adding MPo powder (Figure 2). The color parameters of the stirred flavored yogurt samples supplemented with different amounts of MPo were significantly different ($P < 0.05$; Table 1). Compared with the control, the $L^*$ and $b^*$ values of the 3 groups of MPo-fortified yogurt significantly decreased, whereas their $a^*$ values significantly increased. In MPo-fortified yogurt groups, the $L^*$ and $a^*$ values decreased, whereas $b^*$ increased with the increasing amount of MPo powder. During the 28 d of storage, the color of MPo-fortified yogurt samples kept relatively constant, although their $L^*$, $a^*$, and $b^*$ showed a decreasing tendency. Our results were similar to those flavored yogurts supplemented with other edible anthocyanin-containing plant materials such as pomegranate (Trigueros et al., 2014), blueberry (Felix da Silva et al., 2017), grape pomace (Demirkol and Tarakci, 2018), strawberry pulp (Jaster et al., 2018), and roselle (Levya Daniel et al., 2013). The color changes of the products could be attributed to the rich anthocyanins in MPo. Mature mulberry fruit contains high level of anthocyanins, and MPo contains even higher content of anthocyanins than the whole fruit (Kang et al., 2015). The main anthocyanins in mulberry fruits are cyanidin-3-glucoside, cyanidin-3-rutinoside, and a small amount of pelargonidin-3-glucoside (Qin et al., 2010). After incorporating MPo powder, the pH of the fermented yogurt was below 4.6. As anthocyanins are pH-sensitive pigment components, the main anthocyanins from mulberry turned from red to dark red under this condition (Zhang et al., 2020).

Yogurt has a soft sour taste because it contains organic acids such as lactic acid, which can help humans digest and absorb the nutrients. After adding MPo...
powder, pH values of yogurt samples decreased significantly, and their TA increased accordingly ($P < 0.05$; Figure 3). As there were some organic acids (mainly malic acid and citric acid) in MPo powder, increasing the MPo level more significantly changed the pH and TA of MPo-fortified yogurt samples. During the entire cold storage, the pH of all yogurt samples gradually decreased ($P < 0.05$). The addition of MPo increased the TA of all yogurt samples ($P < 0.05$), and the highest TA value occurred on the 28th day of storage. The slow cofermentation of \textit{S. thermophilus} and \textit{L. bulgaricus} during the storage caused the continuous acidification of the yogurt (Temiz et al., 2014). The organic acids, polysaccharides, and some phenolic compounds in materials added to the yogurt could be served as prebiotics for LAB (Thilakarathna et al., 2018), which caused the additional production of lactic acid and other organic acids compared with the control. The pH changes of these stirred yogurts during cold storage in our experiment were consistent with the results of previous studies (Cano-Lamadrid et al., 2017). The addition of apricot, raspberries, plum, and jamun to yogurt also resulted in decreased pH and increased acidity during storage (Kumar and Kumar, 2016).

### Total Phenolic and Total Anthocyanin Contents of MPo-Fortified Flavored Yogurt

Phenolic compounds are important functional ingredients in MPo-fortified yogurt. We found that incorporation of MPo powder significantly increased the contents of TPC and TAC in yogurt in a dose-dependent manner ($P < 0.05$; Table 2). Phenolic compounds in the developed functional yogurt were derived from MPo, which contained high levels of anthocyanins and other phenolics (Zhang et al., 2011). Our results reflected the extractable phenol content as we used the aqueous-
organic system in this experiment. The addition of other phenolics-rich resources such as anthocyanin-rich purple rice and aronia juice to yogurt also increased the phenolic content of the products (Anuyahong et al., 2020; Nguyen and Hwang, 2016). Interestingly, we found that TPC and TAC contents in the MPo-added yogurt increased gradually and continuously during the later storage period, and reached their highest levels on the last day of storage. The phenolic substances in the MPo powder exist in free and bound forms, and part of the free phenols interacted with milk proteins to form complexes through hydrophilic, charged, or polar AA (Bouarab-Chibane et al., 2018). However, some phenolic compounds bound with polysaccharides or proteins were slowly released during the cold storage; therefore, the contents of extractable phenolics and anthocyanins in the MPo-fortified yogurt gradually increased. Arranz et al. (2009) showed that there was a large number of bound polyphenols in fruits and vegetables, which cannot be directly extracted by the aqueous-organic system. It was reported that cereal fermentation with specific probiotic strains led to significant increase of the content of free phenolic acids (Hole et al., 2012). Microorganisms can metabolize polyphenols into smaller phenolics by their enzyme system. Polyphenols added before fermentation resulted in more extractable phenolic compounds than that added after fermentation in yogurt systems (Sun-Waterhouse et al., 2012). Also, the addition of MPo to yogurt affect protein structure, and the polymer network and gel matrix formed protect anthocyanins from degradation (Sun-Waterhouse et al., 2013), which indirectly increased polyphenol content during yogurt storage. However, the change of anthocyanins in the MPo-fortified yogurt was inconsistent with the results reported by Trigueros et al. (2014), who found that the content of monomeric anthocyanins decreased during cold storage of yogurt with added pomegranate juice. This is most likely because pomegranate juice mainly contains free phenolic compounds, including anthocyanins, and was added to milk before fermentation in their research. As polyphenols have antioxidant properties, this is beneficial to enhance the function of yogurt.

### WHC of MPo-Fortified Flavored Yogurt During Storage

The WHC affects yield, sensory evaluation, stability, and texture of yogurt products. The MPo-fortified yogurts had significantly higher WHC ($P < 0.05$) compared with control yogurts (Table 3). The WHC of control and yogurt with 1% MPo showed increasing tendency, whereas that of yogurt samples supplemented with 2% and 3% MPo was kept constant during the 28 d of cold storage. The increasing WHC of MPo-fortified yogurts can be attributed to dietary fiber from MPo and CN aggregation induced by increased acidity and interactions between milk and mulberry polyphenols. The MPo contains high levels of dietary fiber, especially highly esterified pectin and galacturonic acid (Khodaiyan and Parastouei, 2020). Polysaccharides as hydrocolloids increased WHC in both physical and chemical ways. Physically, free water is trapped and confined within the increased network density, and chemically, the hydrophilic nature of hydrocolloids facilitates the link with water molecules. Previously, many other polysaccharides such as pullulan (Kycia et al., 2018), curdlan (Zhao et al., 2020), okra polysaccharide (Xu et al., 2019), *Ulmus davidiana* pectin (Yeung et al., 2019), and low-methoxyl pectin (Khubber et al., 2021) were used to maintain WHC of yogurt. The increase in WHC may also be due to the increasing acidity and CN aggregation caused by lactic acid fermentation in the yogurt samples during storage (Coggins et al., 2010; McCann et al., 2011). The dropping pH weakened the large particle clots formed during the storage of the yogurt samples (Ozcan-Yilsay et al., 2007), causing the yogurt gel containing MPo to shrink during storage and

### Table 2. Changes (± SD) in total phenol content (TPC; mg of gallic acid equivalent per gram) and total anthocyanin (TAC; μg of cyanidin-3-glucoside equivalent per gram) contents of mulberry pomace (MPo)-fortified stirred yogurt during cold storage at 4°C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (d)</th>
<th>TPC</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>1% MPo</td>
<td>0.70 ± 0.13&lt;c</td>
<td>23.29 ± 0.21&lt;c</td>
<td>0.74 ± 0.90&lt;b</td>
</tr>
<tr>
<td>2% MPo</td>
<td>0.74 ± 0.90&lt;c</td>
<td>41.82 ± 0.21&lt;c</td>
<td>2.96 ± 0.13&lt;b</td>
</tr>
<tr>
<td>3% MPo</td>
<td>3.92 ± 0.14&lt;a</td>
<td>79.09 ± 0.27&lt;c</td>
<td>4.16 ± 0.12&lt;a</td>
</tr>
</tbody>
</table>

$^{A–C}$ Means followed by different uppercase letters in the same column are significantly different ($P < 0.05$).

$^{a–c}$ Means followed by different lowercase letters in the same row are significantly different ($P < 0.05$).
increasing the gel strength. Furthermore, the change of WHC may be related to polyphenol-protein interactions, as polyphenols have a high affinity for CN. The covalent or noncovalent interactions between polyphenols and proteins lead to the formation of stable soluble complexes. The interactions promote the strength of the CN network (hydrophobic interaction) and the formation of hydrogen bonds (Oliveira et al., 2015).

**Rheological Properties of MPo-Fortified Flavored Yogurt**

During the 30-min scan, the apparent viscosity of all the yogurt samples decreased with increasing shear rate (Figure 4). These yogurts had the characteristics of shear-thinning fluids. The addition of MPo at 1%, 2%, and 3% (wt/wt) impaired the declining of viscosity of yogurt samples. With the continuous increase of shear force, the weak bonds within the protein network structures were disrupted, and both electrostatic repulsion and hydrophobic interactions between the molecules were weakened. Under the initial shearing, the viscosity decreased rapidly, and then as the shear rate increased, the fluidity of the protein particles after destruction was consistent with the shear rate. The addition of MPo dose-dependently alleviated the degree of decrease in the viscosity. This indicated that the addition of MPo increased the viscosity of the stirred yogurt ($P < 0.05$).

The storage modulus ($G'$) relates to the elastic energy stored in a sample. The loss modulus ($G''$), a portion of the energy consumed by the sample during the deformation period, is a measure of viscosity (Tamine and Robinson, 1999). The loss tangent ($\tan \delta = G''/G'$) represents viscosity of sample. A larger $\tan \delta$ means a higher viscosity, and the sample would be characterized as a fluid; on the contrary, the sample would be characterized as solid. All the yogurt samples had features of a viscoelastic gel with $G' > G''$ at all frequencies investigated (Figure 5). Both $G'$ and $G''$ increased significantly as the frequency increased ($P < 0.05$; Nguyen and Hwang, 2016). All yogurt samples showed more elastic characteristics under low frequency and more viscous characteristics under high frequency.

The $\tan \delta$ of MPo-fortified yogurts exhibited an upward trend when the frequency changed in the range of 1 to 10 Hz. Our results are in agreement with that of Bikker et al. (2000).

We measured the $G'$ and structure recovery of all yogurt samples within 2 h after stirring (Figure 6). The $G'$ of all yogurt samples increased rapidly at the initial stage. The $G'$ of the control and the yogurt containing 1% MPo increased rapidly, whereas that of 2% and 3% MPo yogurt samples tended to be flat in the middle and late periods. The control had the highest $G'$ value, followed by yogurt samples containing 1%, 2%, and 3% MPo, respectively. These results indicated that MPo reduced the connection between proteins in yogurt after shearing. This is beneficial to increase the consistency, and airy and sticky mouthfeel of yogurt.
The MPo-fortified yogurt exhibited lower firmness, similar cohesiveness, and higher consistency and viscosity compared with the control \((P < 0.05; \text{Table 4})\). All the texture parameters of MPo-fortified yogurt increased gradually during the 28 d of cold storage. The addition of MPo decreased the firmness of yogurt, and this trend was intensified with increasing MPo level. Similar results of firmness were previously obtained with the addition of inulin, partially hydrolyzed guar gum, riceberry rice extract, grape extract, and zeaxanthin nanoparticle to yogurt (Paseephol et al., 2008; Felix da Silva et al., 2017; Mudgil et al., 2017; de Campo et al., 2019; Anuyahong et al., 2020). The MPo dietary fiber can bind water and affect the food stability (Staffolo et al., 2004). It can be assumed that the addition of MPo increased the water content of the yogurt sample. Previous reports suggest that the interactions between polyphenols and CN contribute to decreased syneresis of yogurt because of higher amount of water in the gel. This, in turn, leads to the increased softness and the further decrease in firmness of yogurt (Anuyahong et al., 2020).

Cohesiveness relates to the extent of deformability of material before it is ruptured and is related to internal strength of the material structure. The addition of MPo had little effect on the cohesiveness of yogurt. Viscosity is a major parameter for yogurt. The result of viscosity index was consistent with the apparent viscosity data, showing the thickening function of MPo. As mentioned earlier, the interactions between polyphenols and proteins promoted the weak strength of the CN network (hydrophobic interaction) and the formation of hydrogen bonds. The pH of the yogurts decreased gradually during the cold storage. As a result, the weak postacidification may be responsible for the increase in consistency by strengthening of protein-protein interactions and formation of yogurt structure. Furthermore, pectin can stabilize CN aggregates through electrostatic and steric stabilization, thereby forming CN-pectin complexes and stabilizing the yogurt gel network (Liu et al., 2006). The pectin in the MPo increased the compactness of the protein aggregates, which helped to form uniform and stable network structure by linking protein.

Microstructure of MPo-Fortified Flavored Yogurt

All the yogurt samples exhibited a relatively uniform and porous network structure (Figure 7). The micro-
structure of control yogurt (Figure 7a) was different from that of yogurts incorporated with MPo. The surface of the control was rough, the protein connection was uneven, and many voids and pores were seen. The addition of MPo significantly reduced the pore spaces and channels inside the yogurt samples with denser or more uniform protein aggregates and microstructure (Figure 7b, c, and d). Higher doses of MPo made this phenomenon more obvious (Figure 7c and d). With the increasing MPo content, the pores in the sample network decreased from 46 to 36 μm (Figure 7a) to 5 to 10 μm (Figure 7d). The void areas around the CN aggregates may be filled with MPo particles. They bound to the serum channels around the aggregates, reducing whey separation and increasing WHC.

The microstructure of MPo-fortified yogurt is similar to that of stirred yogurt containing extracellular polysaccharides (EPS) produced by LAB (Gentès et al., 2011, 2013). The protein network of MPo yogurt exhibited a higher cross-linked protein gel network than when EPS was present. This may be due to the higher concentration of MPo particles embedded in the protein network than the released EPS. Furthermore, MPo dietary fiber can bind water and increase the WHC of yogurt samples, leading to enhanced interactions within the protein network (Staffolo et al., 2004). In addition, pectin can further stabilize CN aggregates through electrostatic and steric stabilization, thereby forming a CN-pectin complex and stabilizing the yogurt gel network (Liu et al., 2006). Highly methoxyl pectin can bind to free serum and reduce dehydration (O’Shea et al., 2015). It is possible that pectin in MPo bound to free serum, which increased the compactness of protein aggregates to form a more uniform and stable network structure.

**CONCLUSIONS**

Mulberry pomace is a natural source of pigment, antioxidants, and dietary fiber for stirred-type functional flavored yogurt. The addition of MPo changed the
color to pink and dark red; remarkably decreased the $L^*$ and $b^*$ values and increased $a^*$ values; increased the contents of total acids, polyphenols, and anthocyanins; improved the WHC; and enhanced the uniformness of the microstructure of yogurt during the 28 d of cold storage. It also increased the viscosity and consistency of the yogurt, making the yogurt gel network tighter and the structure more stable. During the cold storage, TPC and TAC contents of yogurt supplemented with MPo increased gradually and continuously. Mulberry pomace is beneficial to improve the desirable characteristics of yogurt and increase consumer acceptability. In the future, the role of individual functional component in MPo affecting the quality of MPo-fortified yogurt needs to be studied.

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