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Preparation and Characteristics of Ball-Milled Blueberry Peel Particles and Their Application in Ice Cream

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Abstract: Ice cream is popular but contains high amounts of saturated fats and few health-promoting ingredients. In the presence of xanthan gum (0.25%), the blueberry peel particles prepared through ball-milling treatment (BMP) were used to prepare ice cream containing camellia oil as a fat replacer. BMP possessed smaller particle sizes, larger contact angles, and higher contents of anthocyanin aglycone, as compared with commonly-milled blueberry peel particles. BMP with the smallest $D_{[4,3]}$ value (about 10 μm) and the largest contact angle (66.30°) were obtained by ball-milling the blueberry peel at 15 Hz for 6 h ($BMP_{15\,Hz\,6\,h}$). The ice cream mixes were depicted as linear viscoelastic gel-like solids, and their apparent viscosity, G′ and G′ increased with the increase in $BMP_{15\,Hz\,6\,h}$ concentrations. Ice cream with strong antioxidant activity and good freeze-thaw stability was acceptable and desirable in the presence of less than 0.5% $BMP_{15\,Hz\,6\,h}$.

Keywords: Blueberry peels; Ball milling; Ice cream; Pickering emulsion

1. Introduction

Ice cream is a popular dessert which is made of milk products, fat, sweeteners, emulsifiers, stabilizers and condiments, and has undergone many processing steps including mixing, sterilizing and homogenizing of all the components (namely preparing of ice cream mix), and subsequent aging, freezing, and hardening (Ewert, Schlierenkamp, Fischer, & Stressle, 2019). The ice cream mix exhibits an oil-in-water emulsion, where proteins, fat globules, ice crystals, air bubbles, and particle stabilizers are dispersed in the continuous phase containing milk proteins, sugars, salts, emulsifiers and hydrocolloids, and is responsible for the stability and texture of ice cream (Ewert, Schlierenkamp, Fischer, & Stressler, 2019). However, the ice cream mix contains a pretty amount of saturated fatty acids, so high consumption of ice cream will increase the risk of obesity and cardiovascular disease, and other health problems (Genovese, Balivo, Salvati, & Sacchi, 2022). Therefore, industry and consumers are continuously seeking alternative ingredients to meet the demand of new and healthier ice cream.

To reduce the amount of saturated fat in ice cream, the liquid oil with high contents of natural unsaturated fat, such as sunflower oil (Moriano & Alamprese, 2017), rapeseed oil (Monié, et al., 2023) and peanut oil (Zaaboul, Tian, Borah, & Bari, 2024), has been incorporated into the ice cream mix after they have been structured into oleogels in the presence of Pickering gelators and/or hydrocolloids, which make the rheological behavior of the liquid oil be similar to that of a solid

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crystalline fat. In addition, the by-products peel powder from pitaya (Utpott et al., 2020), pomegranate (Ismail, Hameed, Refaeym, Sayqal, & Aly, 2020) and persimmon (Yoseffyan, Mahdian, Kordjazi, & Hesarinejad, 2024) has also been added into the ice cream mix to produce the functional ice cream with dietary fibers, polyphenols, and/or antioxidants.

Blueberry, considered as "super fruit", possess the capacities of scavenging free radicals and preventing the cardiovascular disease, neurological decline and type 2 diabetes mellitus, which predominantly attributes to their abundant phytochemicals, especially to their rich anthocyanin pigments (Wang, Camp, & Ehlenfeldt, 2012). However, blueberry anthocyanins are mainly located in the peel, which reach up to 90% anthocyanins of the whole fruit, and they are not easy to be extracted owing to the binding of cell walls (Li, Deng, Pan, Luo, & Zheng, 2023). In addition, blueberry peel which accounts for nearly 30% dry weight of the whole blueberries is usually discarded due to its low solubility and immiscibility. Therefore, it is of importance to find an efficient method to utilize the blueberry peel for the development of blueberry planting and processing enterprises.

Ball milling technology has been used for the production of ultrafine powder and foods, wherein the medium balls colliding in the ball mill tank would result in the materials be modified by creating shear force and stress (Zhao, Zhu, Zhang, & Tang. 2015; Wang, Zhang, Devahastin, & Liu, 2020; Cao et al., 2021). The particles with nanometer/submicron scale from fruit peel, such as yam (Chiang, Chen, & Yeh, 2012) and onion peel (Jiang, Ramachandraiah, Wu, Li, & Eun, 2020), have been obtained using a ball milling grinder. Excitingly, the whole red rice materials were media-milled into nano/submicrometer particles that can used as stabilizers for Pickering emulsions with intrinsic antioxidant properties (Lu & Huang, 2019). Therefore, the ball milling technology may be an efficient method to modify the solubility and immiscibility of blueberry peel as to realize their full utilization and to enlarge their scopes of applications.

Camellia oil from tea seeds (*Camellia oleifera* Abel.) is a unique edible vegetable oil with high contents of oleic acid and tea polyphenols, and can reduce blood lipid levels and prevent coronary heart disease (Luo et al., 2019a). In our previous work, camellia oil-based oleogels have been structured in the presence of xanthan gum (Luo et al., 2019a) and been used as fat replacers to prepare functional cookies (Luo et al., 2019b; Pan, Wu, Luo, He, & Luo, 2020) and soft candies (Pan et al., 2022). The aims of this study are to (1) prepare blueberry peel particles using ball milling treatment at low temperature, (2) evaluate physicochemical characteristics of the ball-milled blueberry peel particles, and (3) investigate the possibility of the preparation of ice cream using camellia oil instead of solid fats, and using blueberry peel particles as stabilizers in the presence of xanthan gum.

2. Materials and methods

2.1. Materials and chemicals

Fresh blueberry (Britewell) fruits were provided by Anhui Huiwang Food Co., Ltd. (Hefei, China). Nile red, 3',6'-dihydroxy-5-isothiocyanato-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (FITC), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid (GA), and cyanidin-3-O-glucoside (CGE), were obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China). All other chemicals used were analytical-grade and bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of blueberry peel particles

Blueberry peel was obtained according to our previous work (Li, Deng, Pan, Luo, & Zheng 2023). The dried blueberry peel was ground with a QSB-200 crusher (Yongkang Industry & Trade Co., Ltd., Yongkang, China) for 3 min and passed through a 60-mesh sieve to give the commonly-milled blueberry peel particles (CMP). CMP (4g) was mixed with ZrO_2 at a mass ratio of 1:4, placed into a BM500 cryogenic mill (Anton-paar, Shanghai, China), and ball-milled at 15 Hz or 25 Hz for 1.5 h, 3 h and 6 h, respectively, to give the ball-milled blueberry peel particles (BMP) (Jiang, Ramachandraiah, Wu, Li, & Eun, 2020), denoted as $BMP_{15\ Hz\ 1.5h}$, $BMP_{15\ Hz\ 3h}$, $BMP_{15\ Hz\ 6h}$, $BMP_{25\ Hz\ 1.5h}$, $BMP_{25\ Hz\ 3h}$, and

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$BMP_{25\ Hz\ 6h}$. The blueberry peel particles CMP and BMP, were separately stored at - 18 °C in double Ziploc bags for further use.

2.3. Property analysis of blueberry peel particles

2.3.1. Size distribution

The particle size distribution was determined with a MS 200 laser particle size analyzer (Malvern Instruments Limited, Britain) at room temperature using the wet technique. D_{10}, D_{50}, D_{90}, $D_{[4,3]}$, $D_{[3,2]}$, Span, Φ (cell wall breakage ratio), and specific surface area (SSA) were recorded, wherein, Span = $(D_{90} – D_{10})/D_{50}$, $\Phi = 1 – (1 – 10/D_{50})$ (if $D_{50} \leq 10$ µm, then Φ = 1) (Xiao, Zhang, Fan, & Han. 2017).

2.3.2. Wettability

The wettability was assessed through monitoring the contact angle with an SCA20 contact angle measuring instrument (DataPhysics Instruments GmbH, Stuttgart, Germany) following our previous work (Luo et al., 2018). The contact angle ($\theta_{o/w}$) was determined according to the captured images of the contact sheet surface.

2.3.3. Micromorphology

The microstructure was observed with a high-resolution field emission scanning electron microscope Regulus 8230 (Hitachi Co, Japan). Blueberry peel particles were carefully placed onto the conductive adhesive carbon tape affixed to the sample plate, and then the micromorphology was examined after gold was sprayed on the sample.

2.3.4. Anthocyanin composition

The anthocyanin composition of blueberry peel particles was analyzed with a UPLCOrbitrap-MS system (Thermo Fisher Scientific Instruments Co., Ltd., Shanghai, China) connected with a Waters Accuracy UPLC HSS T3 column (2.1 mm × 100 mm; Thermo Fisher Scientific Instruments Co., Ltd., Shanghai, China), according to our previous work (Li, Deng, Pan, Luo, & Zheng, 2023).

2.4. Preparation of ice cream

The ice cream was prepared according to the method of Samakradhamrongthai et al. (2021) with slight modifications. The ingredients used in the ice cream formula (IC-B) consisted of skim milk, sucrose, skimmed milk powder, camellia oil, xanthan gum and $BMP_{15\ Hz\ 6h}$ (Table 1). Skim milk and camellia oil were placed into a pan and heated to 50 °C in a water bath. Sucrose, skimmed milk powder, xanthan gum and $BMP_{15\ Hz\ 6h}$ were completely premixed, and then were added and continuously stirred for 10 min to form a homogeneous ice cream mix. The ice cream mix was pasteurized at 85 ± 1 °C for 2 min, then homogenized with an FA25 high shear emulsifying machine (Fluko Co, Germany) for 2 min firstly at 60 °C and 10000 rpm, sequentially homogenized at 16000 rpm for 2 min, and then quickly chilled to 4 °C and aged at 4 °C for 24 h to ensure thorough hydration of all ingredients. The aged ice cream mixes were frozen in an ice cream maker (Hubei Dongbei Electromechanical Co. Ltd., Huangshi, China) for 6 min to 4 °C, further whipped for 7 min, and then hardened at -30 °C for 24h to obtain ice cream samples. The ice cream samples were stored at -18 °C for further use. The ice cream added with 0, 0.1%, 0.3%, 0.5% and 1.0% BMP was designated as IC-$B_{0.0\%}$, IC-$B_{0.1\%}$, IC-$B_{0.3\%}$, IC-$B_{0.5\%}$ and IC-$B_{1.0\%}$, respectively.

Table 1. Formulations of ice cream.

Ingredients (g/100 g)	IC-$B_{0.0\%}$	IC-$B_{0.1\%}$	IC-$B_{0.3\%}$	IC-$B_{0.5\%}$	IC-$B_{1.0\%}$
Skim milk	64.75	64.65	64.45	64.25	63.75
Sucrose	14	14	14	14	14
Skimmed milk powder	11	11	11	11	11

Camellia oil	10	10	10	10	10
Xanthan gum	0.25	0.25	0.25	0.25	0.25
$BMP_{15\ Hz\ 1.5h}$	0	0.1	0.3	0.5	1.0

IC-B, the ice cream prepared with different levels of $BMP_{15\ Hz\ 1.5h}$ added, the subscripts behind which expressing the added level of $BMP_{15\ Hz\ 1.5h}$ in the ice cream mix.

2.5. Rheological behavior analysis of ice cream mix

The rheological behavior of the ice cream mix was measured with a DHR-3 rheometer (TA Instruments, Leatherhead, UK) (Pan et al., 2022). The ice cream mix of 2 ml was tiled on the test platform of the rheometer. The gap between the test platform and the fixture with a diameter of 40 mm was set at 1000 µm. The frequency sweep test was measured at a strain of 0.5% and a frequency range of 0.1–10 Hz. The flow measurement was performed at a shear rate of 0.001 - 100 s^{-1}. All procedures were carried out at 25 ◦C.

2.6. Characteristic assessment of ice cream

2.6.1. Overrun, first dripping time, melting rate and firmness

The overrun, first dripping time, melting rate and firmness of the ice cream samples, were measured according to the method of Liu, Wang, Liu, Wu, & Zhang (2018). A 200-mL container was weighed and the overrun percentage was determined as following: overrun% = (weight of ice cream mixes - weight of frozen ice cream) ÷ weight of frozen ice cream × 100%. The first dripping time was defined as the required time (min) for the dripping of the first melted drop of the ice cream. The melting rate (%/min) was calculated as the slope of the linear curve interval of the dripped portion as function of the time. The firmness was gauged with a physical property tester (Stable micro system Co, Britain) at the test speed of 2.00 mm/s and depth of 20 mm, and the firmness value was obtained through Texture Expert software (version 1.20, Stable Micro Systems, UK).

2.6.2. Macro- and micro-structure observation

The air bubbles scattered in the ice cream were observed through a microscope with a digital camera (Nikon Co, Japan) in a bright area. The distribution of the protein and fat granules in the ice cream was observed through an FV1000 confocal laser scanning microscope (Olympus Co, Japan) the help of fluorescent dyes FITC and Nile red, respectively, at the excitation wavelengths of 633 nm and 488 nm (Cheng, Ma, Li, Yan, & Cui. 2015).

2.6.3. Storage physical stability

The changes in the particle size and creaming index (CI) of ice cream samples kept in a 10-mL transparent centrifuge tube with a lid and stored at 4 ± 1 °C, were monitored on the 7th, 14th, 21st and 28th day of storage. The Z-ave of the ice cream droplet was gauged with a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK), wherein the dispersant of water, particle refractive index of 1.520, particle absorption rate of 0.1 and dispersant refractive index of 1.330 were set. The CI value was calculated as following: CI% = (Hs/He) × 100, where Hs stands for the height of the serum layer, and He for the height of the entire ice cream (Luo et al., 2018).

2.6.4. Sensory evaluation

Sensory properties were evaluated according to the method of Liu, Wang, Liu, Wu, & Zhang (2018) with some modifications. On the first and last day of 28-day storage, sensory evaluation was conducted by 30 pre-trained panelists (18 females and 12 males, ages between 20 and 55) from School of Food and Biological Engineering, using a 5-point hedonic scale for five attributes (appearance, texture, taste, flavor, and overall acceptability), where 1 meant “dislike extremely” and 5 meant “like extremely”.

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2.7. Statistical analysis

All tests were performed in triplicate. The results were expressed as mean values ± standard error. Statistical analysis and multiple comparisons between the means were analyzed by one way analysis of variance and LSD test at 5% level of significance using SPSS 17.0 software.

3. Results and discussion

3.1. Effects of ball-milling on physical properties of blueberry peel particles

3.1.1. Particle size distribution

The particle size has important impacts on the wettability and other characteristics of particles. The particle size of BMP was reduced by approximately 10 times due to the low-temperature ball milling treatment, as compared with that of CMP, and it decreased with the increasing ball milling time (Table 2). When the ball milling time increased from 1.5 h to 6.0 h, the D_{10} value of blueberry peel particles decreased from 30 μm to 3.5 - 5.5 μm, D_{50} value from 210 μm to 15.0 - 24.0 μm, and D_{90} value from 550 μm to 40 - 60 μm, $D_{[4,3]}$ value from 260 μm to 12 - 44 μm, and $D_{[3,2]}$ value from 56.5 μm to 8.5 - 11.5 μm, respectively. Similar results of reduced particle sizes were also found by Jiang, Ramachandraiah, Wu, Li, and Eun (2020) for onion peel, and by Xiao, Yang, Zhao, Wang, & Ge (2022) for pomelo peel. With the increase in the ball milling frequency, the particle size increased and then decreased as a whole. As the ball milling time was extended to 6 h, the particle size of blueberry peel particles obtained at 15 Hz ($BMP_{15\ Hz\ 6.0\ h}$) was smaller than that obtained at 25 Hz ($BMP_{25\ Hz\ 6.0\ h}$). $BMP_{15\ Hz\ 6.0h}$ possessed the lowest difference between D_{90} and $D_{50,}$ the highest ratio of $D_{[4,3]}/D_{[3,2]}$, and the largest span value and Φ value, suggesting that $BMP_{15\ Hz\ 6.0h}$ had more uniform particle size distribution and better homogeneity than other blueberry peel particles (Huang, Dou, Li, & Wang, 2018). Especially, $BMP_{15\ Hz\ 6h}$ achieved 100% of Φ and 1.01 m^2/g of SSA, which indicated that they would be high-quality powdery additives due to a strong digestion possibility of nutrient components (Wang, Zhang, Devahastin, & Liu, 2020).

Table 2. Effects of ball-milling frequency and ball-milling time on size properties of blueberry peel particles.

Samples	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	$D_{[4,3]}$ (μm)	$D_{[3,2]}$ (μm)	Span	Φ (%)	SSA (m2/g)
CMP	29.83 ± 3.37^{a}	210.28 ± 2.55^{a}	547.68 ± 31.34^{a}	256.90 ± 8.43^{a}	56.21 ± 0.51^{a}	2.46 ± 0.02^{c}	13.60 ± 0.00^{d}	0.11 ± 0.00^{e}
$BMP_{15\ Hz\ 1.5h}$	5.43 ± 0.06^{b}	23.12 ± 0.31^{b}	60.95 ± 1.44^{c}	29.25 ± 1.02^{d}	11.46 ± 0.13^{b}	3.04 ± 0.01$^{b\ c}$	81.73 ± 0.03^{c}	0.52 ± 0.01^{d}
$BMP_{15\ Hz\ 3.0h}$	4.01 ± 0.01^{c}	17.36 ± 0.05^{c}	50.95 ± 0.26^{d}	23.94 ± 0.31^{e}	8.90 ± 0.00^{c}	2.70 ± 0.02^{c}	92.38 ± 0.02^{ab}	0.67 ± 0.00^{b}
$BMP_{15\ Hz\ 6.0h}$	2.62 ± 0.16^{e}	15.00 ± 0.14^{e}	36.00 ± 0.88^{f}	12.39 ± 0.13^{g}	7.91 ± 0.28^{e}	2.40 ± 0.03	100.00 ± 0.01^{a}	1.01 ± 0.05^{a}
$BMP_{25\ Hz\ 1.5h}$	3.85 ± 0.25^{c}	17.64 ± 3.44^{c}	75.36 ± 6.12^{b}	80.60 ± 4.21^{b}	9.47 ± 0.76^{c}	4.05 ± 0.01^{a}	91.88 ± 0.02^{b}	0.63 ± 0.03^{bc}
$BMP_{25\ Hz\ 3.0h}$	3.70 ± 0.12^{cd}	16.18 ± 1.22^{cd}	48.75 ± 8.31^{d}	43.69 ± 0.97^{c}	8.84 ± 0.53^{cd}	2.78 ± 0.02^{bc}	94.43 ± 0.01^{a}	0.68 ± 0.04^{b}
$BMP_{25\ Hz\ 6.0h}$	3.54 ± 0.01^{d}	15.12 ± 0.11^{de}	39.92 ± 0.31^{e}	18.90 ± 0.38^{f}	8.49 ± 0.05^{d}	2.41 ± 0.02^{c}	96.12 ± 0.01^{a}	0.71 ± 0.00^{b}

CMP, commonly-milled particles of blueberry peel; BMP, ball-milled particles of blueberry peel, the subscripts behind which expressing the ball-milling frequency and ball-milling time, respectively; Span = $(D_{90} – D_{10})/D_{50;}$ $\Phi = 1 – (1 – 10/D_{50})$, if $D_{50} \leq 10$ μm, then Φ = 1; SSA, specific surface area. D_{10}, D_{50} and D_{90}, calculated by the software using the Rayleigh theory, represent 10 %, 50 % and 90 % of total volume at particle distribution, supposing a spherical shape. Means in the same column with different superscript letters are significantly different at $p < 0.05$.

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3.1.2. Surface properties

Generally, the greater the $\theta_{o/w}$ value of the test samples, the higher their surface hydrophobicity is. CMP had a $\theta_{o/w}$ of 37.43°, which is much lower than that (51.10 - 66.30°) of BMP (Figure 1A), which might be ascribed to the smaller particle size and the presence of higher contents of fiber in BMP. Similar results were also described by Yang & Tang (2021) who reported that the contact angles of okara holocellulose were obviously increased and their surface hydrophobicity were largely improved after ball milling treatment. $BMP_{15\ Hz\ 6h}$ with an $\theta_{o/w}$ of 66.30°, are preferentially wetted by water, but they can also be wetted by oil, so they can stabilize the emulsions through a Pickering mechanism (Chen et al., 2018).

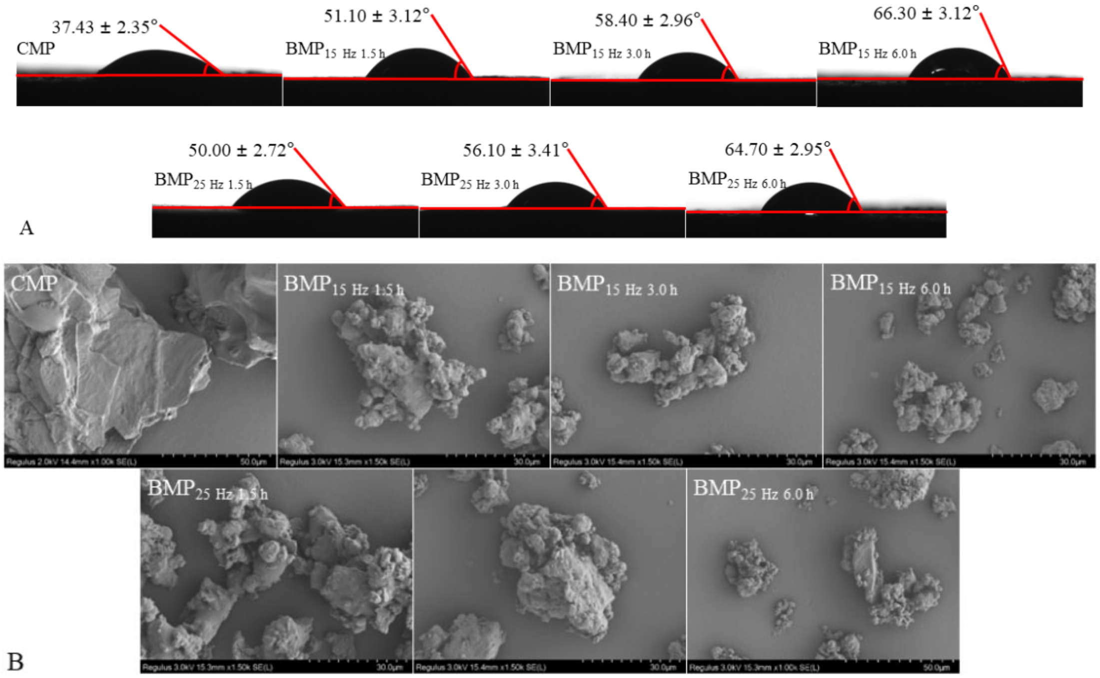

Figure 1. Effects of ball-milling frequency and ball-milling time on contact angle (A) and microstructure (B) of blueberry peel particles.

3.1.3. Microstructure

CMP showed an irregular flaky structure and uneven surface, while BMP exhibited an irregular spherical form (Figure 1B), which suggested that ball milling treatment greatly altered the microstructure of blueberry peel particles. BMP tended to gather in clusters and exhibited a highly agglomerated morphology, which may be due to the small size, large SSA and uniform particles. Wang, Zhang, Devahastin, & Liu (2020) also found that the ball-milled horseradish powder exhibited better morphological properties than other milled samples. Greater modifications in the size and shape of the BMP caused by ball-milling treatment, may bring significant effects on the physicochemical and functional properties of the particles (Table 2 & Table 3).

Table 3. compositions of blueberry peel particles.

No	Anthocyanins	Retention time (min)		$[M-H]^-$ (m/z)	Fragment masses	Percentage (%)	
		CMP	$BMP_{15\ Hz\ 1.5h}$			CMP	$BMP_{15\ Hz\ 1.5h}$
1	**Cy**	**16.83**	16.84	287.05	449.11, 287.06	0.002[b]	0.006[a]

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2	Cy-3,5-O-dig	1.99	1.99	611.16	463.12, 625.18, 301.07	0.005	0.006
3	Cy-3-O-gal/glu	5.57	5.61	449.11	287.06	8.101	8.102
4	Cy-3-O-ara	6.57	6.59	419.10	287.06	2.245	2.245
5	Cy-3-O-sop	11.75	11.74	611.16	317.07	0.099	0.096
6	Cy-3-O-rut	13.05	13.07	595.17	287.06	0.015	0.014
	Total percentage					10.467	10.469
7	Dp	13.22	13.22	303.05	317.07	0.752	0.764
8	Dp-3-O-(6''-p-coumaryl)-glu	16.42	16.42	611.14	303.05	0.070	0.065
9	Dp-3-O-(6-O-acetyl)-glu	14.51	14.53	507.11	303.05	0.373	0.366
10	Dp-3-O-ara	13.22	13.21	435.09	303.05	0.397	0.396
11	Dp-3-O-gal/glu	12.23	12.21	465.10	303.05	1.869	1.872
12	Dp-3-O-rha	5.57	5.58	449.11	317.07	8.064	8.061
13	Dp-3-O-rut	1.99	1.99	611.16	317.07	0.005	0.005
	Total percentage					11.530	11.529
14	Mv-3-O-(6''-acetyl)-gal/glu	10.65	9.92	535.14	331.08, 493.13	1.883	1.885
15	Mv-3-O-(6''-malonyl)-glu	9.51	9.51	579.13	331.08, 493.13	0.138	0.135
16	Mv-3-O-(6-O-p-coumaryl)-O-glu	12.37	12.38	639.17	331.08	0.094	0.092
17	Mv-3-O-ara	8.15	8.16	463.12	331.08	15.248	15.248
18	Mv-3-O-gal/glu	7.42	7.43	493.13	331.08	24.630	24.633
19	Mv-3,5-O-dig	13.09	13.10	655.19	287.06	0.004	0.004
	Total percentage					41.997	41.997
20	Pg	9.07	9.06	271.06	301.07	0.001[b]	0.003[a]
21	Pg-3,5-O-dig	13.05	13.05	595.17	303.05	0.015	0.013
22	Pg-3-O-gal/glu	7.84	7.85	433.11	493.13, 331.08	1.297	1.296
	Total percentage					1.313	1.312
23	Pn	15.76	15.76	301.07	303.05	0.002[b]	0.005[a]
24	Pn-3-O-(6''-acetyl)-gal/glu	10.49	10.44	505.13	331.08	0.265	0.262
25	Pn-3-O-ara	7.84	7.85	433.11	301.07, 463.12	1.297	1.297
26	Pn-3-O-gal/glu	8.15	8.16	463.12	433.11, 271.06	15.248	15.249
27	Pn-3-O-sop-5-O-glu	8.89	8.88	787.23	271.06	0.002	0.001
	Total percentage					16.814	16.814
28	Pt	16.84	16.85	317.06	303.05	0.018[b]	0.025[a]
29	Pt-3-O-rut-5-O-glu	8.89	8.88	787.23	317.07, 479.12, 625.18	0.002	0.001
30	Pt-3-O-(6''-acetyl) gal/glu	9.26	9.27	521.13	301.07	0.887	0.883
31	Pt-3-O-(6''-malonyl)-glu	14.86	14.88	565.12	317.07, 479.12	0.063	0.060
32	Pt-3-O-gal/glu	6.30	6.29	479.12	317.07, 479.12	8.581	8.585
33	Pt-3-O-rut	13.00	12.99	625.18	317.07, 479.12	0.212	0.212
34	Pt-3-O-p-coumaroyl-O-glu	16.65	16.66	625.15	317.07, 479.12	0.010[a]	0.005[b]
35	Pt-3-O-ara	5.57	5.59	449.11	301.07	8.101	8.101

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Total percentage	17.874	17.872

m/z, mass-to-charge ratio; Cy, cyanidin; dig, diglucoside; gal, galactoside; glu, glucoside; ara, arabinoside; sop, sophoroside; rut, rutinoside; Pt, petunidin; Dp, delphinidin; rha, rhamnoside; Mv, malvidin; Pn, peonidin; Pg, pelargonidin. For other abbreviations see Table 2. Means in the same line with different superscript are significantly different at $p < 0.05$.

3.2. Effects of ball-milling on anthocyanin composition of blueberry peel particles

The low-temperature ball milling treatment changed the percentage of individual anthocyanin, while did not change the anthocyanin composition of blueberry peel particles (Table 3). The same 35 anthocyanins in the form of aglycone or glycoside were identified from both CMP and $BMP_{15\ Hz\ 1.5\ h}$, however, $BMP_{15\ Hz\ 1.5h}$ had higher percentage of anthocyanin aglycone and lower percentage of anthocyanin glycoside than CMP. This may be due to the decrease in the particle size and the increase in the surface area of $BMP_{15\ Hz\ 1.5\ h}$, which led to the more extensive hydrolysis of the anthocyanin glycoside by endogenous enzyme of blueberry peel, resulting in the more release of anthocyanin aglycone. Wang, Zhang, Devahastin, & Liu (2020) also reported that low-temperature ball milling treatment accelerated the hydrolysis of substrates (i.e., glucosinolates) and increased the contents of bioactive compounds (i.e., isothiocyanates).

3.3. Effects of BMP on rheological behavior of ice cream mix

With increasing levels of $BMP_{15\ Hz\ 1.5\ h}$, the values of elastic modulus, G′, and viscosity modulus, G″, of the ice cream mix increased at a fixed frequency (Figure 2A). Additionally, G′ and G″ values did not change obviously in the range of the measured oscillation frequency, suggesting that the internal network structure of the ice cream mix was stable and would not be easily changed by the external environment. The G′ value of the ice cream mix was higher than the G″ value over the tested frequency range (Figure 2A & Table 4), indicating that the ice cream mix showed mechanical elasticity. In the ice cream mix, particles, such as fats, are adsorbed on the interface, air bubble and small droplets interact to form a gel-like emulsions (Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013). Meanwhile, when $BMP_{15\ Hz\ 1.5\ h}$ were added into the mix system, they intertwine with other polymers to form a solid-like three-dimensional gel structure, which increased the rheological properties of the mix emulsion. The amelioration of the rheological properties of the ice cream mix emulsion will contribute to the improvement of the viscoelastic structure of ice cream samples (Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013).

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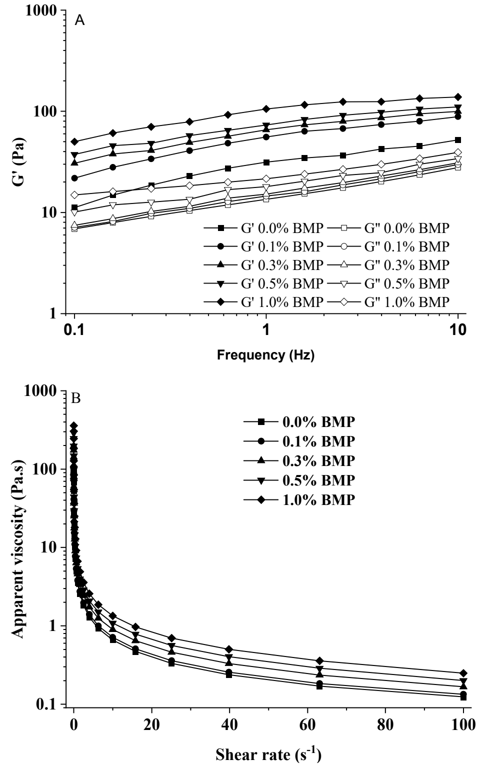


Figure 2. Rheological properties of ice cream mixes with different levels of $BMP_{15\ Hz\ 1.5h}$ added.

The apparent viscosity of the ice cream mix emulsions decreased with increasing shear rate (Figure 2B). The ice cream mix system showed high apparent viscosity at low shear and low viscosity at high shear owing to the breakdown of the internal network and the arrangement of ice crystal s and macromolecules in the shear direction, as well as due to the specific rheological properties of xanthan gum showing the pseudoplastic behavior (shear thinning) (Dogan, Kayacier, Toker, Yilmaz, & Karaman, 2013). The viscosity of the ice cream mixes dramatically increased as the added levels of $BMP_{15\ Hz\ 6\ h}$ increased in ice cream formula (Table 4). This may be due to the introduction of BMP Pickering stabilizers and the increase in the fiber and hydrophilic compound contents of $BMP_{15\ Hz\ 6\ h}$ in the mixture, causing an increase in the apparent viscosity (Soukoulis, Lebesi, & Tzia, 2009). Reducing fat would also lead to an increase of viscosity of ice cream, causing less creamy and harder ice cream (Yu, Zeng, Wang, & Regenstein, 2021). Additionally, the abundant anthocyanins in $BMP_{15\ Hz\ 6\ h}$ might interact with the components of ice cream, such as proteins, unsaturated fatty acid and xanthan gum, finally forming the more stable gel networks, and effectively increasing the viscosity of ice cream (Trigueros, Wojdylo, & Sendra, 2014).

Table 4. viscoelasticity of ice cream mixes with different levels of $BMP_{15\ Hz\ 1.5h}$ added.

Mixes	G′ (Pa s)	G″ (Pa s)	Tanα	G* (Pa s)	η* (Pa s)
IC-$B_{0.0\%}$	31.42 ± 1.77^{e}	13.47 ± 0.78^{cd}	0.43 ± 0.02^{a}	34.19 ± 2.03^{c}	3.52 ± 0.21^{d}
IC-$B_{0.1\%}$	55.65 ± 3.12^{d}	14.53 ± 0.85^{c}	0.26 ± 0.01^{b}	27.52 ± 1.65^{c}	3.80 ± 0.23^{cd}
IC-$B_{0.3\%}$	65.79 ± 3.27^{bc}	15.19 ± 0.85^{c}	0.23 ± 0.01^{bc}	67.52 ± 4.13^{b}	4.59 ± 0.26^{c}
IC-$B_{0.5\%}$	73.33 ± 4.91^{b}	18.02 ± 1.13^{b}	0.25 ± 0.02^{b}	75.51 ± 4.64^{b}	5.38 ± 0.35^{b}
IC-$B_{1.0\%}$	105.53 ± 6.05^{a}	21.56 ± 1.26^{a}	0.20 ± 0.01^{c}	107.71 ± 6.51^{a}	6.68 ± 0.37^{a}

G′, storage modulus; G″, loss modulus; tanα, loss tangent; G*, complex modulus; η*, complex viscosity. Abbreviations see Table 1. Data are expressed as mean ± SD. Means in the same row with different superscript are significantly different at $p < 0.05$.

3.4. Effects of BMP on physico-chemical properties of ice cream

3.4.1. Overrun and firmness

The addition of $BMP_{15\ Hz\ 6\ h}$ significantly changed the overrun and firmness of IC-B samples (Table 5). As the added levels of $BMP_{15\ Hz\ 6\ h}$ increased, the overrun value of the IC-B firstly increased, reaching the highest value of 45.13% (at 0.5 % $BMP_{15\ Hz\ 6\ h}$ addition), then decreased to 38.22% (at 1.0 % $BMP_{15\ Hz\ 6\ h}$ addition) which is still higher than that of the control sample $IC\text{-}B_{0.0\%}$ with an overrun value of 32.99%. The overrun values of blueberry pulp-added ice cream were reported as 36.21 - 39.66% (Sayar, Şengül, & Ürkek, 2022), which is lower than that of $IC\text{-}B_{0.5\%}$. According to the results of Şentürk, Akın, Göktepe, & Denktaş (2024), the addition of 1% - 3% blueberry puree into the probiotic ice cream caused no significant effects on overrun values of samples ($p > 0.05$). Similar to our results, the addition of peanut oil bodies as a fat replacer into ice cream (Zaaboul, Tian, Borah, & Bari, 2024) had a higher overrun value. It has been considered that the overrun value is affected by the contents of fat globule, protein, emulsifier in the ice cream mix, and the viscosity in the production system (Tsevdou et al., 2019). The increased overrun value of $BMP_{15\ Hz\ 6\ h}$-added ice cream could be ascribed to the reduced fat, and the Pickering emulsifier attribute of BMP, as well as the interaction of the abundant anthocyanin in $BMP_{15\ Hz\ 6\ h}$ with proteins and camellia oil.

Oppositely, the firmness of IC-B samples firstly decreased, obtaining the lowest value of 19.03N (at 0.5 % $BMP_{15\ Hz\ 6\ h}$ addition), and then increased to 24.41N (at 1.0 % $BMP_{15\ Hz\ 6\ h}$ addition) which is still lower than that of the control sample $IC\text{-}B_{0.0\%}$ with the highest firmness of 33.15N. The IC-B samples in the present work had lower firmness than the camel milk ice cream with blueberry fruits (137 - 203 N, Sayar, Şengül, & Ürkek, 2022) and the probiotic ice cream with blueberry puree (5000 - 8000 N, Şentürk, Akın, Göktepe, & Denktaş, 2024), but showed similar hardness to the ice cream with persimmon peel (Yoseffyan, Mahdian, Kordjazi, & Hesarinejad, 2024). The firmness of ice cream is a function of the composition in ice cream formulation (protein, fat, sweetener, hydrocolloids, etc.), ice crystal sizes, ice crystal phase volume, system viscosity, air state, and the fat instability (Muse & Hartel, 2004). The compounds that create the network improve the air state (Figure 2), and decrease the ice crystal size (Javidi, Razavi, Behrouzian, & Alghooneh, 2016), therefore reduced the firmness of IC-B samples. Additionally, the firmness of ice creams may ascribe to the overrun. It has been reported that the higher overrun value of ice cream, the less hardness, because the presence of more air in the network of ice cream makes it easy to the penetration of the probe of the texture analyzer (Yoseffyan, Mahdian, Kordjazi, & Hesarinejad, 2024).

Table 5. Physical properties of ice cream prepared with different levels of $BMP_{15\ Hz\ 1.5h}$.

Samples	Overrun (%)	Firmness (N)	First dripping time (min)	Melting rate (%/min)
$IC\text{-}B_{0.0\%}$	32.99 ± 0.85^{c}	33.15 ± 2.16^{a}	14.16 ± 0.93^{c}	3.13 ± 0.82^{a}
$IC\text{-}B_{0.1\%}$	36.51 ± 2.01^{c}	25.97 ± 1.79^{b}	17.43 ± 0.89^{b}	2.84 ± 0.03^{b}
$IC\text{-}B_{0.3\%}$	38.75 ± 2.34^{b}	19.31 ± 1.44^{c}	18.54 ± 1.01^{a}	2.83 ± 0.08^{b}
$IC\text{-}B_{0.5\%}$	45.13 ± 3.06^{a}	19.03 ± 1.18^{c}	20.18 ± 1.07^{a}	2.71 ± 0.02^{b}
$IC\text{-}B_{1.0\%}$	38.22 ± 2.92^{ab}	24.41 ± 1.42^{b}	18.00 ± 1.10^{ab}	2.77 ± 0.11^{ab}

Abbreviations see Table 1. Means in the same row with different superscript are significantly different at $p < 0.05$.

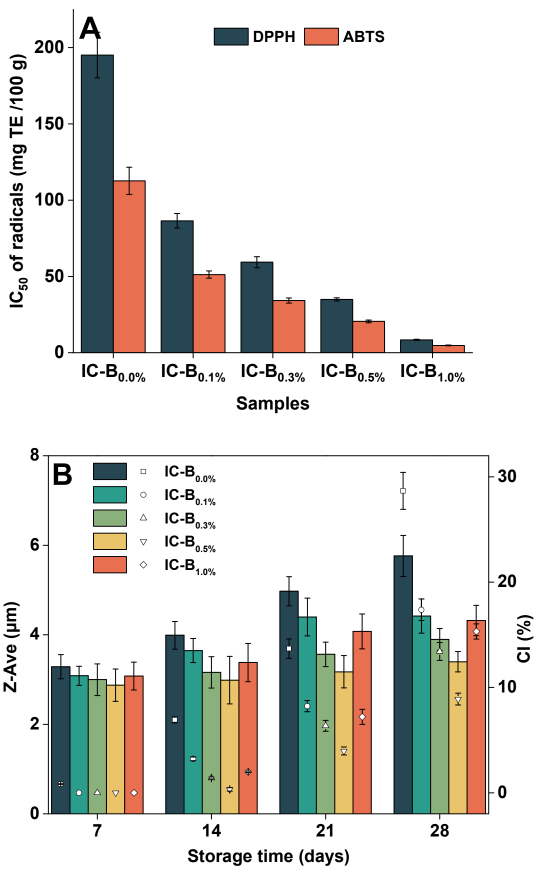
3.4.2. Melting behavior

The addition of $BMP_{15\ Hz\ 6\ h}$ significantly improved the melting resistance of ice cream (Table 5). As the added levels of $BMP_{15\ Hz\ 6\ h}$ increased, the first dripping time quickly prolonged, reaching the maximum value of 20.18 min (at 0.5 % $BMP_{15\ Hz\ 6\ h}$ addition), then slightly shortened to 18.00 min (at 1.0 % $BMP_{15\ Hz\ 6\ h}$ addition) which is still longer than 14.16 min of $IC\text{-}B_{0.0\%}$ sample without $BMP_{15\ Hz\ 6\ h}$. Also, the melting rate of the BMP-added IC-B samples decreased from 3.13 %/min to 2.71 %/min.

Too quick melting loses its edible attribute and is susceptible to thermal shock, while too slow melting is considered as an inferior ice cream product. The meltdown rate reflects both fat stabilization and rheological properties of ice cream, the more stable the fat and the greater the viscosity, the lower the melting rate (Parvar & Goff, 2013; Tsevdou et al., 2019). In the present work, the high viscosity created by xanthan gum and $BMP_{15\ Hz\ 6\ h}$ is responsible for the reduced meltdown rate and the prolonged first dripping time. Similar to our results, the addition of pomegranate peel (Ismail, Hameed, Refaeym, Sayqal, & Aly, 2020) and persimmon peel (Yoseffyan, Mahdian, Kordjazi, & Hesarinejad, 2024) resulted in a significant increase in the melting resistance of ice cream. The first dripping time of camel milk ice cream containing blueberry fruits (26 min - 29 min) were also longer than that of the control without blueberry fruits (23.1 min) (Sayar, Şengül, & Ürkek, 2022).

3.4.3. Antioxidant activities

The addition of $BMP_{15\ Hz\ 6\ h}$ into ice cream formula significantly improved DPPH and ABTS radicals scavenging activities (Figure 3A) of IC-B samples ($P < 0.05$). The IC_{50} values of DPPH and ABTS decreased from 195.13 mg TE/100 g to 8.47 mg TE/100 g, and from 125.22 mg TE/100 g to 5.35 mg TE/100 g, respectively, as the added levels of $BMP_{15\ Hz\ 6\ h}$ increased from 0.1% to 1.0%. Blueberry peel has strong antioxidant activities due to its rich phenolics, anthocyanins and other bioactive substants, and the total phenolics content of which is reported as about 55 mg GAE/g dry weight (Li, Deng, Pan, Luo, & Zheng, 2023). Meanwhile, the contents of anthocyanin aglycons in BMP were further enhanced by ball milling (Table 3). Therefore, the strong free radicals scavenging activities of IC-B samples are attributed to the presence of $BMP_{15\ Hz\ 6\ h}$ that have rich anthocyanins, as well as the antioxidants in camellia oil. The DPPH values of probiotic ice cream with blueberry puree (Şentürk, Akın, Göktepe, & Denktaş, 2024) and camel milk ice cream with blueberry fruits (Sayar, Şengül, & Ürkek, 2022) were reported in the range of 8.91% - 9.20% and 213 - 125 μg/ml (IC_{50}), respectively.



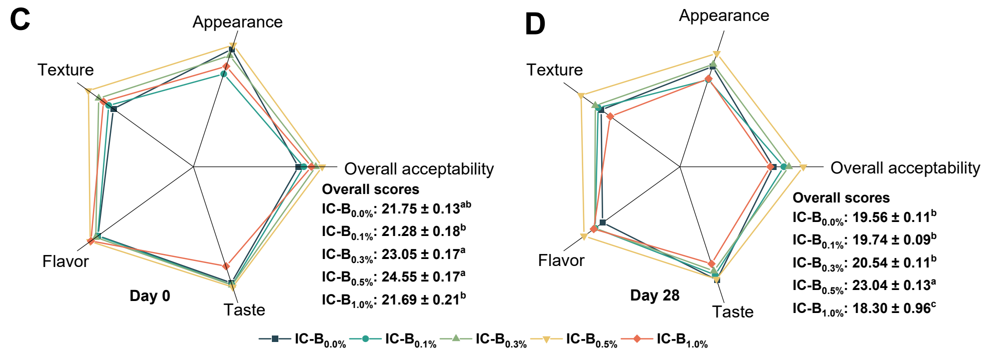


Figure 3. Antioxidant activities, storage physical stability (B) and sensory scores (C & D) of ice cream prepared with different levels of BMP$_{15\ Hz\ 1.5h}$.

3.5. Effects of BMP on storage stabilities of ice cream

3.5.1. Storage physical stability

The storage physical stabilities were determined by Z-ave and CI (Figure 3B). As the added levels of BMP$_{15\ Hz\ 6\ h}$ increased, the Z-ave and CI values of IC-B samples decreased firstly during storage, reaching the lowest values at 0.5 % BMP$_{15\ Hz\ 6\ h}$ addition, and then increased at 1.0 % BMP$_{15\ Hz\ 6\ h}$ addition. The Z-ave and CI values of all IC-B samples increased with the extension of the storage time, but the increment was lower for IC-B samples with BMP$_{15\ Hz\ 6\ h}$. Also, after 7 days of storage, there was no significant difference in Z-Ave and CI values for all of IC-B samples with BMP$_{15\ Hz\ 6\ h}$, nevertheless, with the extension of the storage time, IC-B$_{0.5\%}$ showed the lowest values of Z-Ave and CI values. The results suggested that a suitable level of BMP$_{15\ Hz\ 6\ h}$ added was required for the preparation of ice cream with high storage stability, which is in line with the results of Velasquezz-cock et al. (2018), and that IC-B$_{0.5\%}$ showed the highest storage physical stability.

3.5.2. Sensory attributes

IC-B$_{0.0\%}$ and IC-B$_{0.5\%}$ scored the highest appearance ($P > 0.05$), which showed tempting creamy yellow and blueberry purple, respectively. No significant differences in the taste scores were observed among the samples, except for IC-B$_{1.0\%}$. Compared with IC-B$_{0.0\%}$, the texture scores of IC-B$_{0.1\%}$, IC-B$_{0.3\%}$, IC-B$_{0.5\%}$ and IC-B$_{1.0\%}$ increased by 5.80%, 18.21%, 31.13% and 12.14%, respectively. The variation trend of the flavor and overall acceptability scores was similar to that of the texture score. Similar to our results, ice cream incorporated with appropriate amount of blueberry puree (Sayar, Şengül, & Ürkek, 2022; Şentürk, Akın, Göktepe, & Denktaş, 2024) or fruit peel (Ismail, Hameed, Refaeym, Sayqal, & Aly, 2020; Yoseffyan, Mahdian, Kordjazi, & Hesarinejad, 2024) could be desirable to consumers.

During storage, as the added levels of BMP$_{15\ Hz\ 6\ h}$ increased, the overall score value of IC-B samples increased firstly, reaching the highest value of 24.55 (at 0.5 % BMP$_{15\ Hz\ 6\ h}$ addition), and then decreased to 21.69 (at 1.0 % BMP$_{15\ Hz\ 6\ h}$ addition), which is similar to that of IC-B$_{0.0\%}$ (Figure 3B). On the last day of storage, the overall score of all samples decreased, while IC-B$_{0.5\%}$ had the highest overall score, followed by IC-B$_{0.3\%}$ IC-B$_{0.1\%}$, IC-B$_{0.0\%}$ and IC-B$_{1.0\%}$ (Figure 3D), which agrees with the results of Z-ave and CI (Figure 3B) and suggested that the addition of 0.5% BMP$_{15\ Hz\ 6\ h}$ in ice cream mix may be a threshold level that is appealing for consumers. This may be because 0.5% BMP$_{15\ Hz\ 6\ h}$ not only give the ice cream an intense color and various functional compositions (Table 3 & Figure 4), but also improved the texture due to its Pickering particles attribute which helped the air bubbles, protein and fat globules distributed evenly (Figure 4).

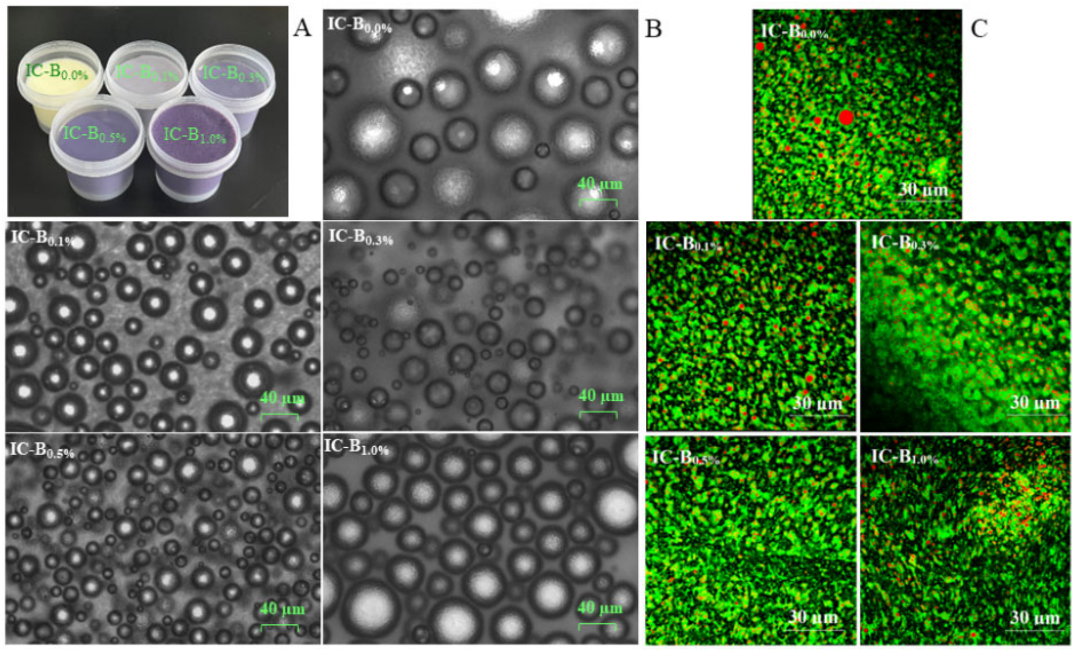


Figure 4. Visuals (A), optical microscopy images (B) and confocal micrographs (C) of ice cream prepared with different levels of BMP$_{15\ Hz\ 1.5h}$.

3.6. Effects of BMP on macro-and micro-structure of ice cream

The color of IC-B samples changed from milky yellow to deep purple red as the added level of BMP$_{15\ Hz\ 6\ h}$ increased from 0 to 1.0% (Figure 4A), wherein purple (at 0.5 % BMP$_{15\ Hz\ 6\ h}$ addition) is the enjoyable color. The air bubbles in the IC-B$_{0.0\%}$ sample were big and sparse, which occurred probably due to the high fat content (10%) of camellia oil; nevertheless, more and smaller air bubbles were observed as the addition amount of BMP$_{15\ Hz\ 6\ h}$ increased to 0.5%, but the air bubbles became too big as the added level of BMP$_{15\ Hz\ 6\ h}$ was 1.0% (Figure 4B). Many red big fat globules in the IC-B$_{0.0\%}$ sample could be seen in the visual field, but the fat globules gradually diminished and evenly distributed as the added level of BMP$_{15\ Hz\ 6\ h}$ was increased up to 0.5%, however, the size of the fat globules became big again (Figure 4C). Similar results were found by Bilbao-Sainz, Sinrod, Chiou, & McHugh (2019) who added strawberry powder into the ice cream mix and found that an appropriate addition level was necessary for the uniform distribution of air bubbles and fat globules in ice cream and delectability of products. BMP$_{15\ Hz\ 6\ h}$ ameliorated the distribution of air bubbles and fat globules in ice cream through the following ways: (1) BMP$_{15\ Hz\ 6\ h}$ might serve as Pickering emulsifiers for enhancing the interfacial properties of ice cream, (2) abundant dietary fiber with colloidal properties could stabilize oil in water, (3) abundant phenolics and anthocyanins acted with protein further increase the viscosity, and (4) the pectin BMP and xanthan gumin have the power to reinforce the gel structure, preventing bubble collapse in the process.

4. Conclusions

The blueberry peel particles with Pickering attributes were obtained by low-temperature ball-milling treatment at 15 Hz for 6 h, and were used for the preparation of ice cream containing camellia oil. BMP$_{15\ Hz\ 6\ h}$ with a $D_{[4,3]}$ of 12.39 ± 0.13 μm and a $D_{[3,2]}$ of 7.91 ± 0.28 μm possessed an $\theta_{o/w}$ of 66.30° and high contents of anthocyanin aglycone. The ice cream mixes with different levels of BMP$_{15\ Hz\ 6\ h}$ added showed the linear viscoelastic solids exhibiting pseudoplastic flow, and behaved as a strong gel-like dispersion with G′ much greater than G″. As the addition of BMP$_{15\ Hz\ 6\ h}$ in the ice cream formula was increased from 0% to 1.0%, the overrun value of ice cream changed from 32.99% to 36.51 - 45.13%, onset time from 14.16 min to 17.43 - 20.18 min, melting rate from 3.13 %/min to 2.71 - 2.84 %/min, and firmness from 33.15 N to 19.03 - 25.97 N, and their antioxidant activity, freeze-thaw stability and storage stabilities were improved. The ice cream added with no more than 0.5% of $BMP_{15\ Hz\ 6\ h}$ was acceptable and desirable.

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Conflicts of Interest: The authors declare no conflict of interest.

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