

Physicochemical Characteristics of the Functional Dairy Dessert Containing Encapsulated Red Beet Extract

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ABSTRACT: In the present study, red beetroot extract was encapsulated within a double emulsion and added to a dairy dessert. In the first part, the effect of red beetroot extract added to the inner aqueous phase of the W/O/W double emulsion was studied by some properties such as droplet size, zeta potential, physical stability, pH, color, turbidity, viscosity, and microstructure. After that in part 2, different dairy dessert samples (control dessert, dessert containing double emulsion, dessert containing free beetroot extract, and dessert with double emulsion containing beetroot extract) were prepared and analyzed for 30 days of storage time. The formation of double emulsion systems was confirmed by the microscopy technique. The droplet size of the control double emulsion and the double emulsion containing red beetroot extract was 3.47 μm and 4.27 μm , respectively. The results showed good antioxidant (51.94 %) and anti-microbial activities for the dairy dessert containing encapsulated extract during the storage time. The pH of this sample did not change after 30 days and its sensorial properties showed good overall acceptance. While increase in the microbial count and decreasing in the pH and antioxidant activity were observed for the dairy dessert containing the free extract. These results showed that the application of fortified double emulsion can improve the quality of cream and other oil products.

KEYWORDS: Antioxidant activity, Dairy dessert, Double emulsion, Encapsulation, Red beet extract.

INTRODUCTION

Beetroot with the scientific name *Beta vulgaris* L. is mainly used in fresh or canned forms or as juice due to its high nutritional benefits. Red beetroot has high concentrations of different phenolic compounds including ferulic, vanilic, p-hydroxybenzoic, and coumaric [1]. The other micronutrient group present in the red beetroot is its pigments known as betalainins, which have strong anti-oxidant properties. Betanin as one of the significant

categories of betalainins in the beetroot, is the only allowed pigment to be applied in the foods [2,3]. Betanin is mainly located in the peel of the red beetroot, acts against plant pathogens, and as an antioxidant compound. It is approved that the beneficial effects of betanin on the human body would exist even after digestion. In a study, it was shown that the amount of indicaxanthin in plasma serum was sufficiently high even 180 minutes after passing through the intestine

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and had a significant anti-inflammatory effect on a model system. [4]. In addition to the direct usage of red beetroot, European people use its juice [5]. High concentrations of pigments and phenolic compounds in the pomace of red beetroot after juicing make it a valuable by-product [6].

Double Emulsion (DE) is a type of emulsion system in which, a dispersion of a single emulsion within a second (outer) continuous phase. Oil in Water in Oil (O/W/O) and Water in Oil in Water (W/O/W) are two types of DE systems. In the latter form, Water in Oil (W/O) emulsion is added to a continuous aqueous phase [7]. Different types of oil phases can be used in these systems. From the nutritional perspective, unsaturated oils are proffered in this regard as compared to the saturated types. However, the sensitivity of unsaturated oils to oxidation makes their usage limited [8]. One strategy to overcome this problem is to use antioxidant agents in the oil phase of W/O/W double emulsion [9]. Due to the consumer's demand and health issues, nowadays antioxidants derived from natural sources are preferred to synthetic types [10, 8]. Natural antioxidants from plants have phenolic compounds in their chemical structures which give them the ability to prevent oxidation [11,12]. Encapsulation of different food extracts such as pomegranate peel, pomegranate, and sea buckthorn (*Hippophae rhamnoides* L.) pomace in stable double emulsion is one of the main today's research projects. [13-15]. Application of this encapsulated system can improve the quality properties of novel food products by high oil content.

Based on our knowledge there aren't any research on the encapsulation of red beet extract in double emulsion and also application of it in the dairy dessert. In this research, we prepared W/O/W double emulsion and used red beet extract in the inner aqueous phase as an antioxidant agent. Then, the stability and physicochemical properties of the emulsion were examined. In the next step, double emulsions with and without the extract were added to the dairy dessert and physicochemical and microbial aspects of the dessert were evaluated.

EXPERIMENTAL SECTION

Material

Olive oil (Ferico, Iran), red beetroot, bovine gelatin (Beyotime Company, China), wheat starch (Mashhad starch Company, Iran), sugar, and milk (Pegah, Iran) were purchased from local markets in Shiraz, Iran. Polyglycerol polyricinoleate (PGPR), sodium chloride, and Tween 80 were prepared from Merck Co, Germany.

Beetroot extract preparation

For this purpose, red beetroot was cut into small pieces (1*1 cm), and 50 g of it was added to 100 mL double distilled Water (DDW), and heated at 70 °C under continuous shaking. After, half an hour, the obtained extract was collected and filtered using a Whatman filter paper (No. 1) and finally evaporated using a rotary evaporator to 60% of its dry weight [16]. The concentrated beetroot extract was then kept in frozen form until use.

LC-MS of extract

A Perkin-Elmer API 165 (Norwalk, CT, USA) single quadrupole MS instrument with Turbo-Ionspray interface was used for Liquid Chromatography (LC) ElectroSpray Ionization (ESI) Mass Spectrometry (MS) analysis. Scan spectra in the negative and positive ion modes were determined in the range of 150 amu to 1000 amu. ESI settings were: temperature 350°C ~ ion spray 4500V, curtain gas 8psi, CEM detector 2300V in negative mode. The MS detector was coupled to Hewlett Packard (Palo Alto, CA, USA) 1100 HPLC system consisting of a high-pressure mixing pump, autosampler, column oven, and DAD. On-line UV spectra were determined in 220–500 nm. The solution of red beetroot extract in formic acid was prepared and then it was mixed with distilled water and acetonitrile at a 1:5:94 volume ratio and centrifuged at 13000 g for 180 s. Then, 5 µL of supernatant was removed and injected into the instrument. Eluent A and B were distilled water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid, respectively; at the rate of 500 µL/min for linear gradient elution [17].

Double emulsion preparation

W/O/W double emulsion was prepared in two steps based on the method of Kumar and Kumar [18]. The inner aqueous phase was composed of distilled water, red beet extract (at a certain concentration to supply 100 mg of phenolic compounds in the final product), and sodium chloride (0.6% w/w). The mixture of PGPR and olive oil heated at 50 °C for 25 min composed the middle oil phase. These two phases were mixed with a 2:8 (aqueous phase to oil phase) mixing ratio to obtain the primary water in the oil emulsion and then added to the outer aqueous phase (a 4:6 mixing ratio), which was composed of distilled water, Tween 80 (6% w/w), and sodium chloride (0.6% w/w) heated at 50 °C for 60 min.

Emulsion characterization

Particle size

Static Light Scattering (SLS) was used to determine the mean droplet size and distribution of the prepared emulsion by a Malvern Particle Size Analyzer (Model: Malvern, Mastersizer v3.50). The refractive index of the diluted sample was 1.33. The volume means diameter and distribution (based on volume density) of the sample were reported as the results. The experiment was performed at 20 °C and the sample was diluted 100 times with distilled water before the test [19].

Zeta potential

The surface charge of the double emulsion droplets was determined by a Zetasizer Nano-ZS90 (Malvern Instrument Ltd., Malvern, Worcestershire, UK) at 20 °C. The sample was diluted 100 times with distilled water before the experiment [20].

Turbidity

The turbidity of the prepared double emulsion was determined by a spectrophotometer (UV-1650PC, Shimadzu, Japan) at 600 nm. Deionized distilled water was selected as the blank. The sample was diluted 500 times with distilled water before the experiment [21].

Physical Stability

To determine the centrifugal (physical) stability of the double emulsion, the sample was centrifuged at 5500 g and 5 °C for 10 min. Then, the volume of the serum phase was determined [21]. In the below equation to calculate the stability:

$$\% \text{ physical (centrifugal) stability} = (\text{volume of W/O/W} - \text{volume serum}) / \text{volume of W/O/W} \times 100$$

pH

A pH meter (Starter 3000, OHAUS, Switzerland) was used to determine the pH of the prepared sample at 25 °C [22].

Color

To determine different color parameters of the prepared double emulsion including L*, a*, and b*, the sample was placed in a wooden box lightened with a 6500 K Lamp. Images were captured from the sample using a camera (Canon Powershot A540, 6 megapixels resolution) while the camera was placed at a vertical 25 cm distance from the samples. Adobe Photoshop® CS6 software was used to calculate the mentioned parameters [20].

Encapsulation efficiency and release of red beetroot extract

Encapsulation Efficiency (EE) of red beetroot extract within the inner aqueous phase of the W/O/W emulsion was determined based on the method of Hemar *et al.*, [23]. Equation (1) was used to calculate EE as below:

$$EE (\%) = (C_{w1} - C_{w2} / C_{w1}) \times 100 \quad (2)$$

Where C_{w1} and C_{w2} are the concentration of the initial polyphenol added to the inner aqueous phase and the concentration of polyphenol measured in the outer aqueous phase, respectively.

Tannin at different concentrations in the inner aqueous phase of double W/O/W emulsion (at the same condition with the difference that red beetroot extract was substituted by tannin) was used to obtain a standard curve at 725 nm to determine the number of polyphenols present in the beetroot extract in the outer aqueous phase. Double emulsion without red beet extract was considered blank. The release of red beetroot extract from the inner aqueous phase of the double emulsion to the outer aqueous phase was measured based on the method described by Gharehbeiglou *et al.* [24].

Viscosity

To determine the rheological behavior of the samples, a rheometer (Anton-Paar, MCR52, Anton Paar, Ostfildern, Germany) with a cone and plate geometry (CP-75) (1.002° inclination and 0.149 mm gap) was used. The shear rate was changed from 0 to 226 1/s in 3 min during the experiment. Viscosity and shear stress were calculated by the software (Rheoplus/32, Service V.3.61). The experiment was carried out at 25 °C and repeated three times [19].

Microstructure

To study the microscopic structure of the prepared double emulsion, an optical microscope (Biovis BA 310, Expert Vision Labs Private Limited, India) was used based on the method explained by Kumar *et al.* [18]. One drop of sample was put on a glass slide and a cover slip was placed on it so that no air bubble was observed between the sample and slip. The slides were then examined by the microscope 100X lens with immersion oil between the sample slide and it.

Dairy dessert preparation

Dairy dessert was produced based on the formulation suggested by Zare and Lashkari, [22]. Bovine gelatin

Table 1: Polyphenol component of red beet extract

Polyphenols	Retention time (min)	Concentration (mg/g dw E)
Gallic acid	0.25	0.01
Ferulic acid	0.33	0.93
Chlorogenic acid	0.81	0.02
Caffeic acid	1.01	0.47
Vanillic acid	1.17	1.96
Syringetic acid	1.26	0.83
Quercetin	1.59	3.12
Myricetin	2.01	2.09
Kampferol	2.35	3.75
Ellagic acid	2.44	2.55

(0.5% w/w), wheat starch (4% w/w), sugar (0.5%), and milk were mixed and then red beetroot extract in the free form/encapsulated (in the double emulsion) form was added (5% w/w) to the previous mixture and heated for 15 min at 95 °C and then mixed using a high shear homogenizer (Ultra-Turrax T45) at 70 °C for 240 s at 10000 rpm. Then samples were kept in the refrigerator overnight before analysis. Each dessert sample was prepared three times.

Dairy dessert characterization

pH

pH value of dessert samples containing free and encapsulated red beetroot extract was measured by Zare *et al.* at 25 °C [22].

Antioxidant activity

Antioxidant activity of dessert samples containing red beetroot extract was examined by measuring radical scavenging activity using, 2-diphenyl-1-picrylhydrazyl free radicals (DPPH°) based on the method of Hashemi Gahrue *et al.* [17] with slight modifications. For this purpose, different amounts of each dessert sample were added to methanol to prepare 0.0125-0.2 mg/mL. Then, each diluted sample was added to 1.5 mL of DPPH° solution and kept in darkness for 60 min at room temperature. The absorbance of samples was measured at 517 nm. Blank was the sample without any added dessert.

Microbial properties

Microbial analysis of dessert samples including yeast count, mold count, and total count was determined as described by Kaur and Goswami [25] after the production of samples.

Sensorial properties

The produced dessert samples were analyzed by twelve semi-trained panelists for their flavor, color, odor, sourness, and overall acceptance. Five-point hedonic scale (5 = very good, 4 = good, 3 = middle, 2 = bad, and 1 = very bad) was used for this experiment.

Statistical analysis

All values were expressed as mean \pm SD. The results were analyzed by one-way ANOVA. IBM SPSS Statistics 20 software was used to determine the statistical significance between groups (in part 1: control emulsion and fortified emulsion; part 2: creams incorporated with emulsion). A significant difference was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Red beetroot extract composition

Table 1 shows the result of the LC-MS analysis of red beetroot extract. It can be seen that the polyphenol compounds present in the extract were Kampferol, Quercetin, Ellagic acid, Myricetin, and Vanillic acid at 3.75 mg/g, 3.12 mg/g, 2.55 mg/g, 2.09 mg/g, and 1.96 mg/g concentrations, respectively. The total concentration of polyphenol compounds in the red beet extract was 15.84 mg/g. In another research, it was reported that the main polyphenol compounds presented in red beetroot extract were 4-hydroxy benzoic acid, cinnamic acid, vanillic, chlorogenic, trans ferulic acid, and caffeic acid [26]. The presence of different types of flavonoids (quercetin, kampferol, and myricetin) and phenolic acids (ferulic, vanillic, syringic, and caffeic) in the root and stem was approved by Ben Haj Koubaier *et al.* [27]. The amount of total phenolic compounds was reported 22.5 mg/mL in the red beetroot extract [28]. Georgiev *et al.* [29] approved the presence of 4-hydroxyl benzoic acid and caffeic acid in the beetroot.

Emulsion properties

Particle size, Zeta potential, Stability, and pH

The particle size of an emulsion is influenced by several parameters such as emulsification method and emulsifier type and affects the encapsulation efficiency and stability of the emulsion. In this research, a two-step emulsification method was used to fabricate a W/O/W double emulsion containing red beetroot extract in the inner aqueous phase. Based on Fig. 1a, the droplet size of the control double emulsion and the double emulsion containing red beetroot extract were 3.47 μ m and 4.27 μ m,

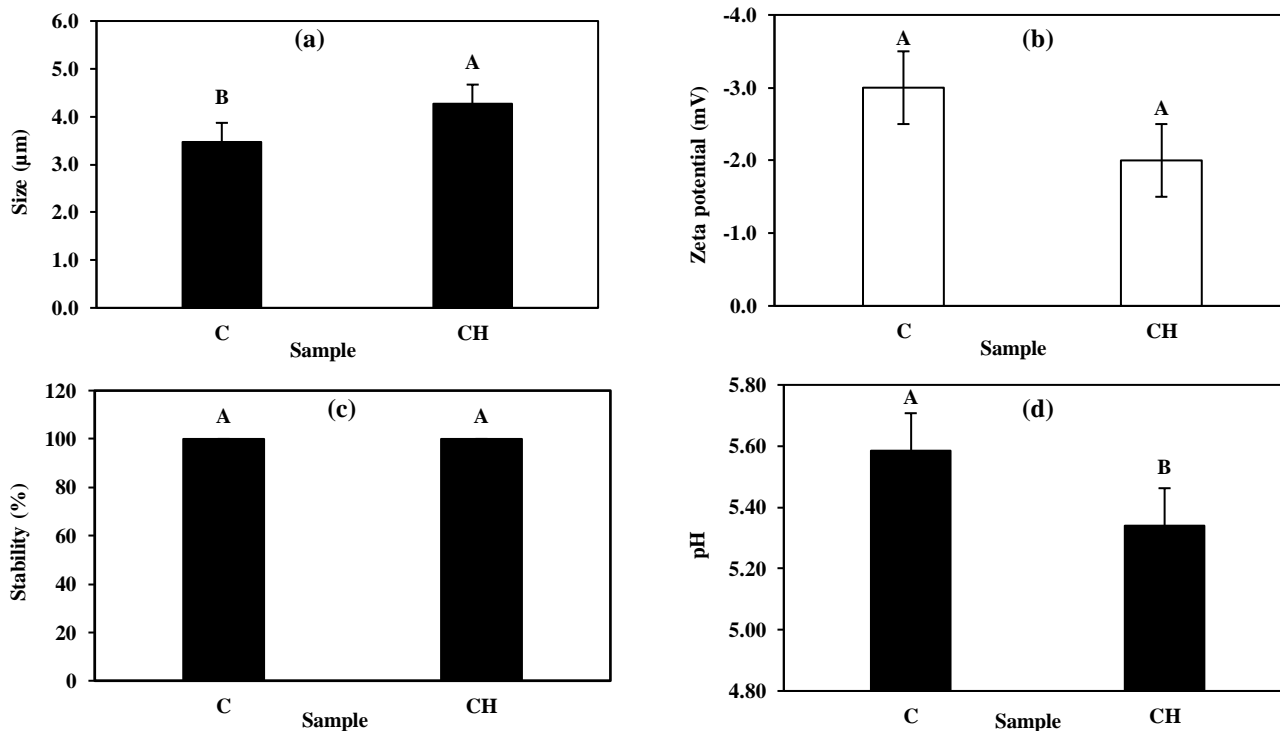


Fig. 1: Droplet size (a), zeta potential (b), stability (c), and pH (d) of control double emulsion (C), and red beet extract fortified double emulsion (CH). Mean \pm SD within a column with the same uppercase letters is not significantly different at $p < .05$

respectively. As can be observed, the presence of red beetroot extract increased the droplet size probably due to the high viscosity of the extract and also particles with different sizes in the extract. In another work, a droplet size of 5 μm was reported for double emulsion encapsulating betanin [30]. Based on the report of *Aditya et al.* [31], the size of W/O/W emulsion droplets with and without curcumin and catechin ranged from 2.82 μm to 3.88 μm .

Fig. 1b shows the zeta potential of the samples. The surface charge of the control and fortified W/O/W double emulsions were -3 mV and -2 mV, respectively. It can be seen that the presence of red beetroot extract did not affect the zeta potential, significantly. A similar result was reported by *Aditya et al.* [31]. They showed that fortifying double emulsion with curcumin and catechin did not change the surface charge of the droplets. Emulsion stabilized by non-ionic surfactants such as Tweens and Spans should theoretically have no charge. But, in practice, they often have a low negative charge due to the presence of free fatty acids or other ionic impurities [32].

The physical stability of the control and fortified double emulsion was examined and the results are presented in Fig. 1c. It was observed that both samples were completely

stable (100%) without any phase separation and oiling off, one day after production, which means that they have the potential to be used in the food systems.

The result of pH measurements that are presented in Fig. 1d indicated a lower pH value for the double emulsion containing red beetroot extract (5.34) as compared to the control sample (5.59). It can be related to the presence of different types of acidic compounds in the red beetroot extract.

Viscosity

Viscosity results of the control and fortified double emulsion systems (Fig. 2a) showed higher viscosity in the fortified emulsion (135.39 cP) compared to that one (133.45 cP). That can be related to the presence of concentrated high-viscose extract. Fig. 2b shows that the two samples had a non-Newtonian behavior so their viscosity decreased by increasing the shear rate. This shear-thinning behavior is the result of breaking the bridges between the droplets at higher shear rates and rearrangement of the emulsifier molecules in the rotation direction so, fewer interactions between molecules. A similar result was reported by *Hosseini et al.* [19].

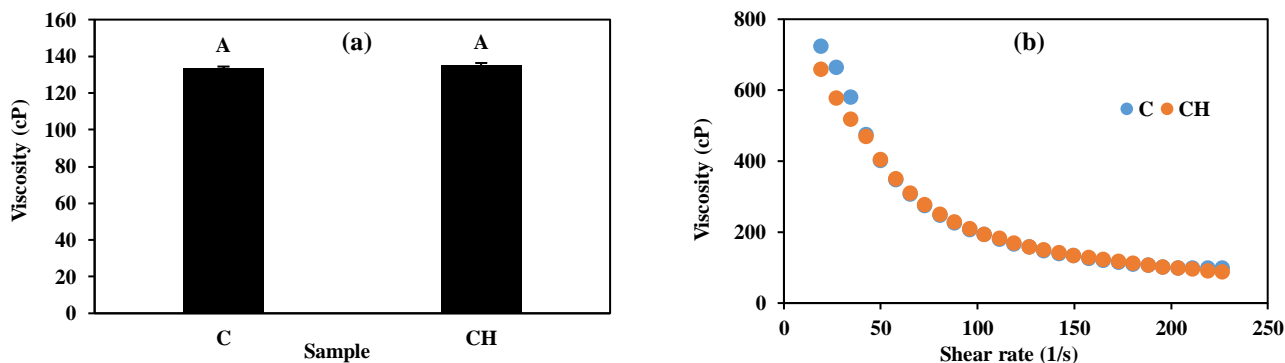


Fig. 2: Viscosity (a) and non-Newtonian behavior (b) of control double emulsion (C), and red beet extract fortified double emulsion (CH). Mean \pm SD within a column with the same uppercase letters is not significantly different at $p < .05$

Color and Turbidity

Fig. 3 shows the color parameters including lightness (L), redness-greenness (a), and blueness-yellowness (b) of the control and fortified double emulsions. As can be seen, in the presence of red beetroot extract, lightness decreased and redness and yellowness increased significantly. This is due to the presence of red pigments in the red beetroot extract. Fortifying double emulsion with the extract did not affect turbidity significantly and both samples showed similar absorbance at 600 nm.

Microstructure

The microstructures of control and fortified W/O/W double emulsions are presented in Fig. 4. The presence of an inner aqueous phase within oil droplets dispersed in an outer aqueous phase can be observed in the figures, which confirms the formation of W/O/W double emulsions. Similar results were revealed in the research of *Kaimainen et al.* [30]. There aren't any differences between the microstructure of the two samples.

Dairy dessert properties

pH

The result of pH measurements of different dessert samples including the control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free red beetroot extract (CH) are presented in Fig. 5. The dessert sample with free red beetroot extract showed lower pH value compared to other samples due to the presence of different acidic compounds in the extract. Similar to our results, *De Moura et al.* [33]

reported a lower pH value for yogurt after adding encapsulated Hibiscus extract. During 30 days of the storage time, the pH value of the control dairy dessert and the dairy dessert containing double emulsion without red beetroot extract decreased significantly, while the pH values of other samples did not change. This result can be related to the activity of microorganisms such as lactic acid bacteria in the two mentioned samples and also can be evidence of the antibacterial properties of red beetroot extract.

Antioxidant activity

The results of antioxidant activity of control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free extract (CH) during storage are presented in Fig. 6. As can be seen, in the presence of red beetroot extract in the dairy desserts, the samples showed high anti-oxidant properties. On the first day of storage, the antioxidant activity of the sample containing free extract was higher than that containing encapsulated extract, which can be due to the greater availability of the free extract to interact with the DPPH^o. However, during the storage time (after 15 days and 30 days) the antioxidant activity of the sample containing free extract decreased considerably because of the susceptibility of the antioxidant components to environmental conditions including the acidic pH of the sample, oxygen, and the presence of other active compounds. Similar results were reported by *De Moura et al.* [33]. *Oliveira et al.* [34] reported that encapsulation of strawberry preparation containing polyphenol compounds within bigger protein particles protected it better than encapsulation within small particles. In addition, the antioxidant activity of strawberry preparation decreased by 24% one day

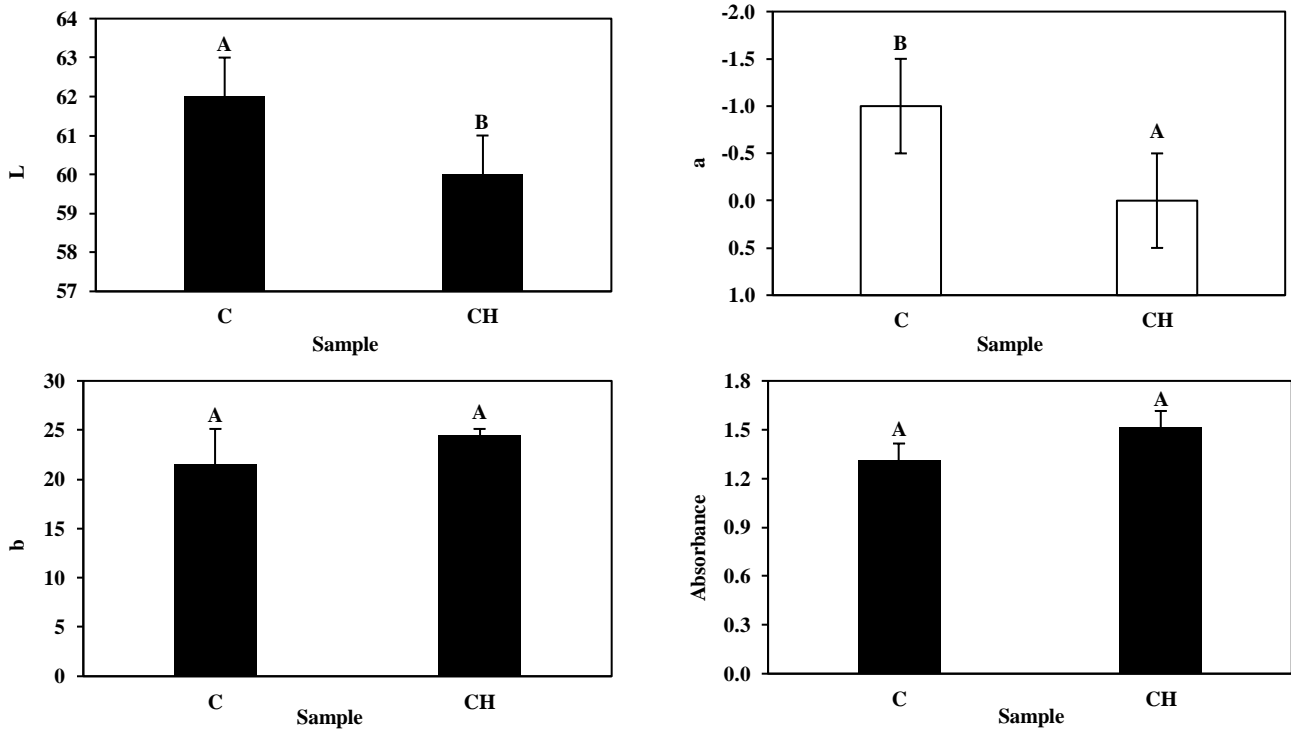


Fig. 3: Color and turbidity of control double emulsion (C) and red beet extract fortified double emulsion (CH). Mean \pm SD within a column with the same uppercase letters is not significantly different at $p < .05$

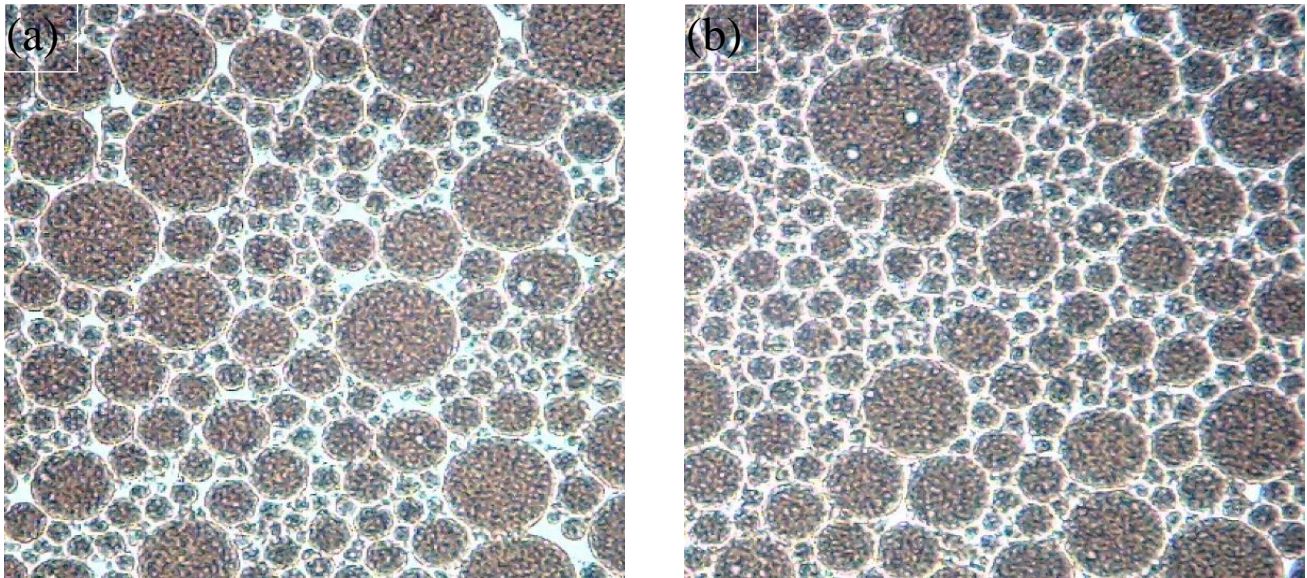


Fig. 4: Microstructure of control double emulsion (a) and red beet extract fortified double emulsion (b).

after adding it to the yogurt sample. The results of other research showed that free anthocyanin was very sensitive to pH and protein and was not stable in the milk. In yogurt, anthocyanin could interact with proteins. The resultant complex could protect anthocyanin, however, its bioavailability decreased [35].

Microbial properties

The results of the microbial count of different dessert samples are indicated in Fig. 7. It was observed that the total count as well as yeast and mold count of the samples containing red beetroot extract decreased during the 30 days of storage time, while the microbial count of

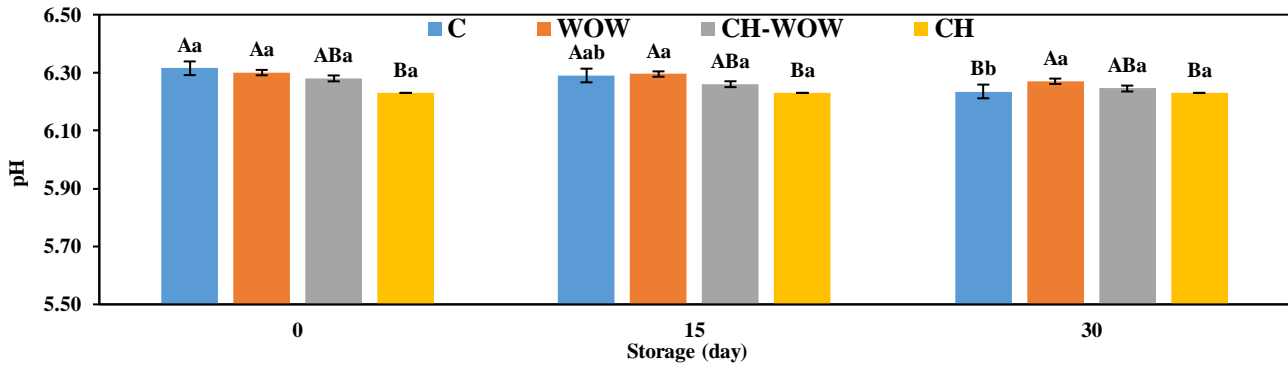


Fig. 5: pH of control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free extract (CH) during storage. The same uppercase letters between treatments and the same lowercase letters between different times are not significantly different at $p < 0.05$.

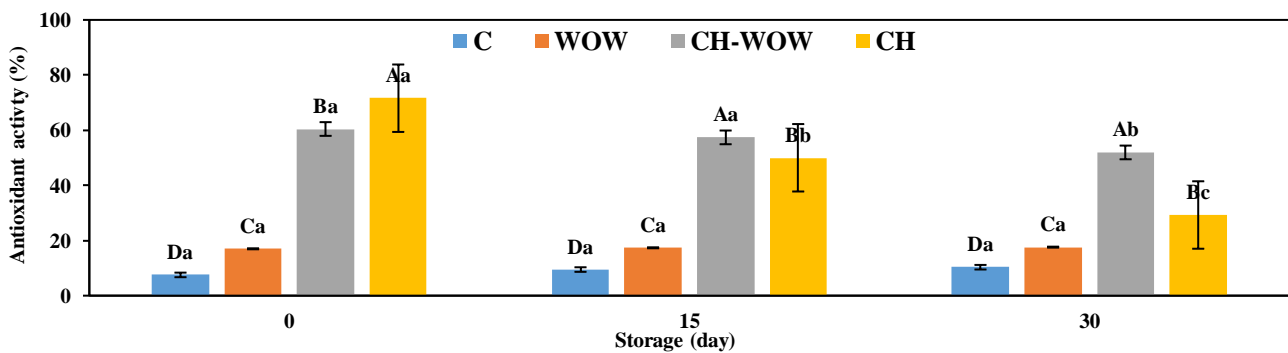


Fig. 6: Antioxidant activity of control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free extract (CH) during storage. The same uppercase letters between treatments and the same lowercase letters between different times are not significantly different at $p < 0.05$.

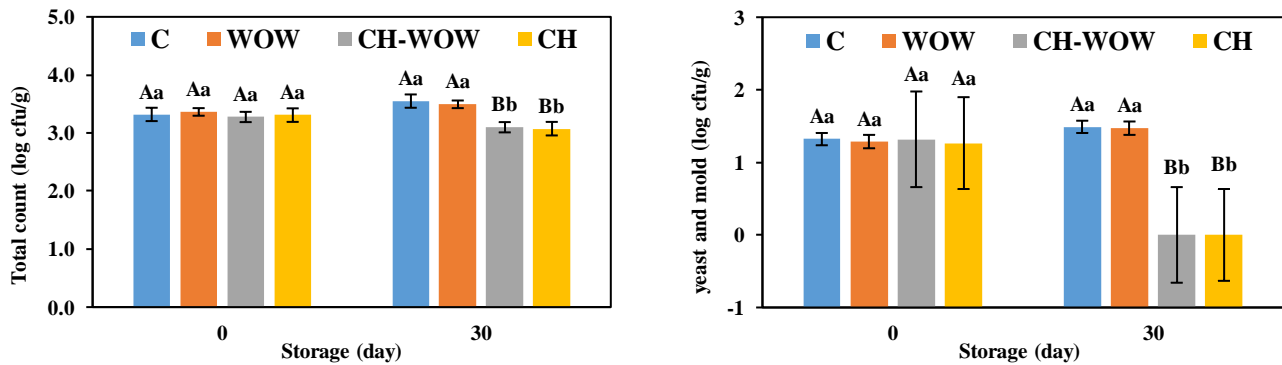


Fig. 7: Total count and yeast and mold count of control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free extract (CH) during storage. The same uppercase letters between treatments and the same lowercase letters between different times are not significantly different at $p < 0.05$.

the control dairy dessert and the dairy dessert containing double emulsion without the extract increased. This result confirmed the antimicrobial activity of red beetroot extract (as seen before in the section Antioxidant activity). The antimicrobial activity of red beetroot extract was also observed by Vulić *et al.* [36] and Kumar and Brooks [37].

Sensorial properties

Sensorial properties of different dairy dessert samples including color, odor, taste, sweetness, hardness, and overall acceptance were examined and the results are presented in Fig. 8. Adding un-encapsulated (free) red beetroot extract to the dairy dessert decreased the taste, sweetness, and overall

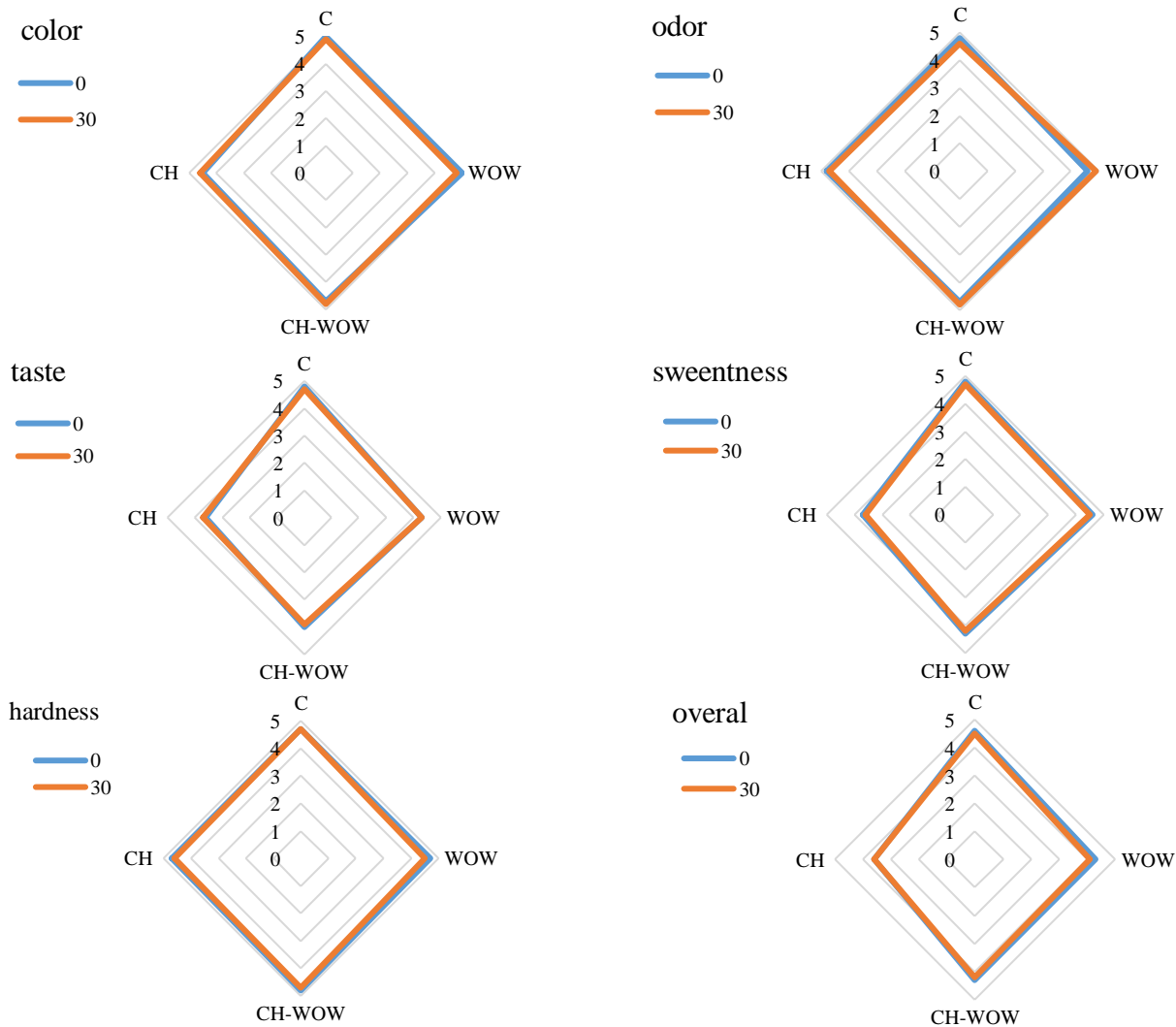


Fig. 8: Sensorial properties of control dairy dessert (C), dairy dessert containing control double emulsion (WOW), dairy dessert containing fortified double emulsion (CH-WOW), and dairy dessert containing free extract (CH) during storage.

acceptance scores, while encapsulation of the extract did not affect the color, odor, and hardness of samples containing the free and encapsulated extract. Samples containing encapsulated extract had higher scores in terms of taste, sweetness, and overall acceptance than samples containing free red beetroot extract. *Keshvari Koohejani and Lashkari (2022)* reported similar results with our finding of adding free and encapsulated iron to the sensory properties of cream [21]. The sensorial aspects of the dairy dessert samples did not change during the 30 days of storage.

CONCLUSIONS

In this study, the novel W/O/W double emulsion incorporated with red beetroot extract in the inner aqueous phase was prepared. Different parameters of the prepared

double emulsion containing the extract including droplet size, zeta potential, physical stability, turbidity, and viscosity as well as examining the microstructure showed the appropriateness of this system as a carrier for red beet extract and also applying it to fortify dairy dessert. The results showed that this fortified double emulsion had good stability (size, zeta, and physical stability) and efficiency. The dairy dessert containing encapsulated red beetroot extract (within W/O/W emulsion) showed considerable antioxidant activity at the beginning and end of the storage time. The microbial count of this sample decreased during the time due to the antimicrobial effect of the extract and the pH value did not change. While increase in the microbial count and decreasing in pH and antioxidant activity were observed for the dessert containing the free

extract. The overall acceptance of the dessert with encapsulated red beetroot extract had an acceptable score. Based on the obtained results, W/O/W double emulsion is a good candidate to encapsulate red beetroot extract and fortify dairy desserts.

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