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ORIGINAL PAPER

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MICROENCAPSULATION OF NATURAL POLYPHENOLIC COMPOUNDS EXTRACTED FROM APPLE PEEL AND ITS APPLICATION IN YOGHURT

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ABSTRACT

Background. Apple peel is a by-product of fruit processing and a rich source of natural antioxidants, especially of polyphenolic compounds. Although it has many health benefits, the microencapsulation of polyphenolic compounds protects it from reactions with milk components during manufacturing or storage of dairy products which reduce the bioavailability and total acceptability of these products.

Materials and methods. Polyphenolic compounds (PC) were extracted from apple peel using ethanol (80%). Polyphenolic compounds extract powder (PCEP) was encapsulated by physical methods (spray and freeze dryer) using maltodextrin, whey protein concentrate (8:2), and Gum Arabic mixture (6:4) as coating materials, which were homogenized by ultraturrax and ultrasonication. Encapsulated PCEP was used in supplementing yoghurt. Phenolic content (PC), physiochemical and texture properties of yoghurt samples were evaluated during storage (fresh, 7 and 15 days).

Results. The microencapsulation by freeze dryer method for PCEP which was homogenized by ultrasonication was the best treatment, while encapsulation efficiency using the spray dryer method, which was homogenized by ultraturrax, was the worst. Encapsulated PCEP in yoghurt samples didn't have any significant influence on the physiochemical and texture properties of these samples.

Conclusion. Yoghurt samples maintained on the polyphenolic compounds until the end of storage overall, our results revealed that adding encapsulated PCEP into yoghurt gave closer characteristics to the control sample.

Keywords: apple peel, polyphenolic compounds, microencapsulation yoghurt, spray and freeze dryer

INTRODUCTION

Apples (*Malus pumila*) are used commonly in human nutrition due to their availability throughout the year and a pleasant taste. They also contain many nutrients, such as vitamins, minerals and other bioactive components. Peels and seeds are the major by-products of fruit processing and they contain high amounts of phenolic compounds (PC), flavonoids and antioxidants compared to whole fruit (Ajila et al., 2007; Vieira et al., 2009). Natural phenolic compounds are receiving a lot of attention due to their potential beneficial health properties (Bueno et al., 2012). An apple has 80% of its PC concentrated in the peel, and five-to-six-fold the antioxidants in the apple peel than in the flesh (Leccese et al., 2009). The increasing health awareness of consumers has created a great demand for functional foods containing PC (Sun-Waterhouse

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et al., 2010; 2012a; 2013a). Yoghurt is one common dairy functional food that presents dietary delivery systems for PC. It is known that PC are sensitive ingredients and their application in foods presents challenges due to their stability whether during food processing or storage (Sun-Waterhouse et al., 2010; 2012a; 2013a). Encapsulation technology can solve this problem, where encapsulation protects bioactive ingredients like PC from damage during food processing (Sun-Waterhouse et al., 2012b). Freeze and spray-drying are the most applied physical encapsulation techniques, both of which depend on water being removed, either by sublimation of the frozen product or by conversion of the performance of the product from solution to powder using a continuous feeding process in a hot drying medium, respectively (Gharsallaoui et al., 2007). The advantage of the freeze-drier technique is that the product components aren't exposed to high temperatures, which can maintain the nutritious characteristics of the product. On the other hand, the spray-drying technique is very suitable for any product in a liquid state (Do Espírito Santo et al., 2013). It is important to choose the coating materials which are used in the encapsulation process because it may affect encapsulation efficiency and the capsule's stability. There is a wide range of coating materials used in the encapsulation process, such as dextrin, Gum Arabic, gelatin, starch and whey protein (Fang and Bhandari, 2010). The most common coating material used in encapsulating is maltodextrin (MD) because it has a low bulk density and viscosity, is easy to form films from, and creates a barrier to oxygen (Rai et al., 2016). Another polysaccharide commonly used in encapsulation processes is Gum Arabic because it has a high capacity for forming a good emulsifier and film (Silva et al., 2013). Whey protein (WP) is a by-product of the dairy industry which is commonly used in the food industry because it has a high nutritional value and the physiochemical properties of (emulsion, stabilization and gel formation).

The aim of this study is production of yoghurt supplemented with encapsulated PC extracted from apple peel by using two different encapsulation techniques: freeze and spray-drying.

MATERIALS AND METHODS

Materials

Apples (Amasya) were bought from a local market in Istanbul, Turkey. Skimmed milk powder (low heat), was made in the USA. Starter strains of *Streptococcus thermophiles* and *Lactobacillus delburkii* ssp. *bulgaricus* were obtained from stock cultures of a Microbiology laboratory of the Food Engineering Department in Istanbul Technical University, Turkey. Maltodextrin, whey protein concentrate (80%) and Gum Arabic were purchased from Alfasol Co., Turkey. Folin-Ciocalteu reagent, Gallic acid and 1,1-diphenyl-2-pycrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co. (St. Louis, USA). All solvents were used in HPLC grade.

Preparation of apple peel

Apples were selected free from bruises and visual defects then washed with flowing water and peeled. The apple peel was collected and stored in a freezer (–18°C) until used. The method of Grigoras et al. (2013) was used for preparation of the apple peel extracts with slight modifications. 1 g of freeze dried and ground powder of apple peel was extracted by 20 ml ethanol (80%) and exposed to an ultrasonic bath at 25°C/20 min. All extractions were centrifuged at 5000 rpm for 10 min and the supernatant removed. A rotary evaporator (Büchi R20, Switzerland) was used at 40°C until the volume decreased and all ethanol was removed. The treatments were repeated three times, then the concentration was frozen at −20°C overnight and then dried using a freeze dryer (Christ Alpha 1-2LD plus, Germany). The temperature of the ice condenser and the vacuum pressure were set to −50°C and 0.04 mbar, respectively. After running the freeze-drying process for 72 h, the dried product was collected, pulverized and stored in an airtight container for further analysis.

Identification of phenolic and flavonoid compounds

UHPLC analysis. A Shimadzu LC-10A apparatus (Kyoto, Japan) equipped with a SPD-M10A photodiode array detector (PDA) was used for analytical UHPLC separations. Reversed-phase chromatography

was performed with a 250 × 4.6 mm Kromasil 100 C-18 column packed with 5 µm particles (teknokroma, Barcelona, Spain), fitted with a security guard C18 ODS (4 × 3.0 mm i.d.). Gradients were formed with a He-degassed solvent. Solvent A was H2O containing 0.1% formic acid, and solvent B was MeCN by applying different elution conditions. Separation was accomplished starting with 5% A for 2 min at pressure of 115 bar, followed by a linear gradient performed for 10 min from 5% B to 95% A and subsequent linear gradients from 20% to 95% A in 5 min. The flow rate was 0.5 ml·min^{-1}, and the operating temperature was 40°C. The injection volume was 10 µl. The chromatogram was recorded at 286 nm.

Encapsulation procedure. Polyphenolic compounds extract powder – PCEP was encapsulated with three different coating materials. The first wall material was MD : WPC at ratio 8 : 2 then PCEP was added (1 : 20). An Gum Arabic mixture (second wall material) was added to the previous mixture at ratio 4 : 6. This mixture was exposed to two different homogenization methods: high-speed homogenizer (ultraturrax) at 4000 rpm for 5 min and ultrasonication (160 W power, 20 KHz frequency and with 50% pulse, Sonic Ruptor 400, OMNI International the Homogenizer Company, GA, USA). Then the mixtures were dried using two methods: freeze drying for 48 h and spray drying. The spray dryer (March Cool Industry Co., Ltd., Bangkok, Thailand) was operated at an inlet temperature of 150°C and an outlet temperature of 50°C. For the freeze-drying process, the mixtures were placed in freezing trays and frozen at −20°C immediately after preparation. After 24 h, the frozen mixtures were dried for more than 48 h at −45°C under a pressure of less than 0.120 mbar using a freeze dryer (Model 7948030, Labconco, USA). The spray-dried and freeze-dried samples were kept at −18°C for further analysis. At the time of use, the dried content was ground into a fine powder. Each experiment was triplicated.

Encapsulation characterizations

Encapsulation efficiency (EE). Encapsulation efficiency (EE %) was calculated by determination phenolics content (PC), antioxidant activity (AA) and total flavonoids (TF) before and after encapsulation. Five grams of sample were dissolved in ethanol (70% v/v) for 5 min and the quantities of TPC, AA and TF were determined as mean values and triplicate measurements (Ades et al., 2012; Fernandes et al., 2014).

Phenolics and flavonoids content. Phenolics content (PC) was measured using a Folin-Ciocalteu assay (Wollgast and Anklam, 2000) while flavonoids content (FC) was recorded according to the method described by Lee et al. (2003). The results were expressed as mg of catechin equivalents (CE) per kg of defatted samples. All samples were analysed in triplicate.

DPPH-radical scavenging activity assay. DPPH--radical scavenging activity was determined according to the method introduced by Kumaran and Karunakaran (2006) and Rai et al. (2006). 0.1 mM of DPPH was prepared by dissolving 3.943 mg DPPH in 100 ml ethanol. 100 µl of each sample was mixed with 2 ml methanolic solution of DPPH (0.1 mM). Extracts were substituted with methanol and distilled water blanks. Decolorization of purple free radical DPPH solution was measured at 517 nm after 30 min incubation in the dark and at room temperature. The antioxidant activity was calculated by using the following equation:

$$\text{Antioxidan activity, \%} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100$$

Surface morphology analysis. Particle structures of PCEP capsules were evaluated by scanning Electron Microscopy (Quanta FEG 250 SEM; Thermo Fisher Scientific, Oregon, USA), which was used to acquire the morphology of microcapsule powder at an accelerating voltage of 10.0 kV. Polyphenolic compound extract powder – PCEP microcapsule samples were sprinkled onto an adhesive coated aluminum pin stub. The stubs were sputter coated with a thin layer of gold in a Leica vacuum coating unit at 40 mA for 100 s 3 times, at a working distance of 50 mm by using an argon gas purge (Quorum SC7620 Sputter Coater). Digital images were taken at a magnification of 4000×, and 12,000× (Ferreira et al., 2007a).

Measurements of particle size distribution and zeta potential. The particle size and zeta potential

were determined with a dynamic light scattering instrument (Nano ZS, Malvern Instruments, Worcestershire, UK).

Yoghurt preparation. Skimmed milk powder (12%) was reconstituted in distilled water. Polyphenolic compound extract powder was used to replace total solids of SMP at ratios 5, 10, 15 and 20%. All samples were subjected to pasteurization (85°C for 30 min) and cooled directly to 45°C before being inoculated with starter bacteria (*S. thermophiles* and *Lb. delburkii* ssp. *bulgaricus*) at 3%. The previous samples were incubated at 42°C until the curd formed then stored in a refrigerator at 5 ±2°C.

Physicochemical characteristics of yoghurt

pH and titratable acidity measurement. pH was measured by a pH meter (JENWAY 3505) equipped with a combined electrode. Titratable acidity of the yoghurt was measured according to the 942.15 AOAC (2000), and results were expressed as lactic acid %.

Color measurement. Hunter LAB (Color quest XE, Hunter Lab, USA) was used to analyze the color of yoghurt samples. Illuminant D65 was the source of light and the observation was 10 degrees. The color value was evaluated as L^* (lightness), a^* (negative value means green and positive value means red), and b^* (negative value means blue and positive value means yellow). All samples were done in triplicate.

Apparent viscosity. A dynamic viscometer (Brookfield Model-LV; Brookfield Engineering Laboratory, Stoughton, USA) was used at a speed of 100 rpm. All the assays were performed in triplicate.

Statistical analyses

The data obtained in this study was expressed as the mean of triplicate determinations. Statistical comparisons were made with Duncan's test which was analyzed with SPSS (SPSS for Windows, Version Rel. 10.0.5, 1999, SPSS Inc.). P-values < 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Identification of phenolic and flavonoid compounds by UHPLC

The HPLC method was used to determine the individual PC from the apple peel. Sixteen phenolic compounds used as standards are listed, with retention times and absorbance ratios. All the standards used were determined by their spectral properties Table 1 and Figure 1. The highest amount of PC in the apple peel was phloridzin (321.28 mg·kg^{-1}). Balázs et al. (2012) and Jakobek et al. (2013) stated that flavanols and anthocyanins are characteristic compounds of apple peel. The total value of identified PC was 1141.92 ppm and this can be confirmed by Jakobek et al. (2013) who mentioned PC in apple peel ranged from 672 to 3150 mg·kg^{-1}.

Table 1. UHPLC of PC identified in apple peel

	Phenolics group	Retention time	Concentration of phenolic compounds mg/kg
1	4-hydroxybenzoic acid	10.328	0.55
2	Gallic acid	13.99	120.03
3	3,4-dihydroxybenzoic acid	15.68	8.46
4	Unknown	15.756	
5	Catechin	16.151	88.27
6	Rutin	16.496	77.20
7	Chlorogenic acid	17.003	68.24
8	Unknown	17.067	
9	Unknown	17.201	
10	Ferulic acid	18.058	102.51
11	Caffeic acid	18.224	126.27
12	Phloridzin	18.568	321.28
13	p-coumaric acid	18.795	0.62
14	Ethyl 3,4-dihydroxybenzoat	19.531	18.21
15	Quercetin	19.604	93.90
16	t-cinnamic acid	20.866	116.38

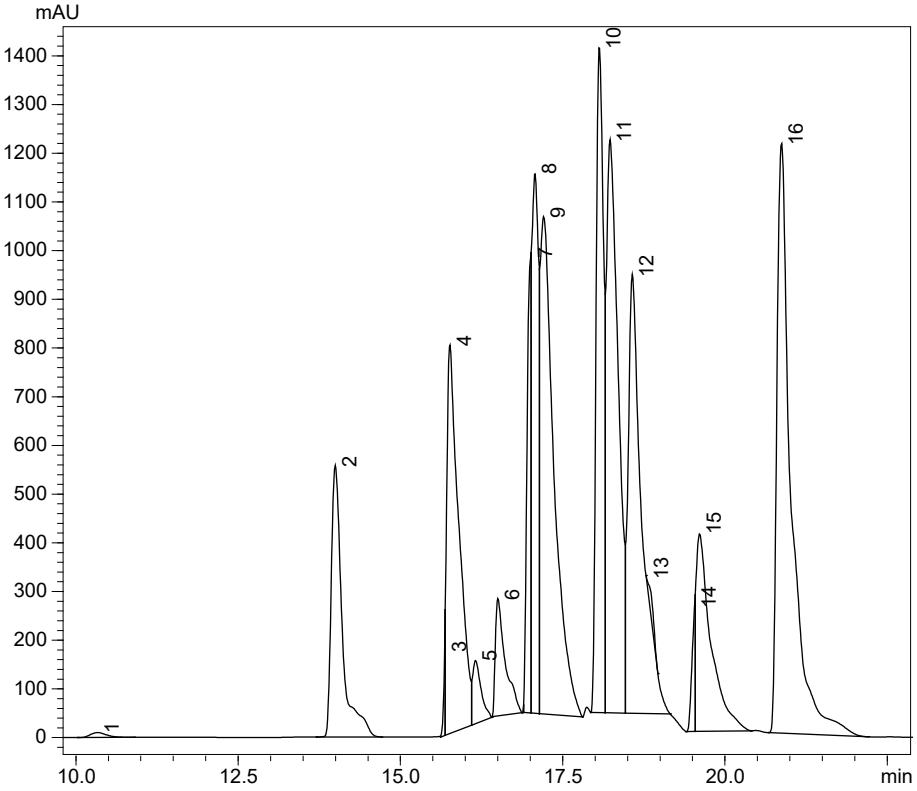

Fig. 1. UHPLC of PC identified in apple peel

Table 2. Particle size and zeta potential of encapsulated PCEP

Treatments	Particle size, n	PdI	Zeta potential, mV
Ultraturrax	719.07 ±61.554	0.441 ±0.013	–27.633 ±0.208
Ultrasonic	315.500 ±26.212	0.416 ±0.038	–28.833 ±0.153

Particle size and zeta potential

Table 2 shows the particle size and zeta potential for PCEP and the wall materials mixture which was homogenized by ultrasonic and ultraturrax. The results of particle size and zeta potential referred to use ultrasound for 20 min in encapsulation let to decrease the size of capsules than capsules formed by ultraturrax and this is due to increase the energy density and time sonication where lead to form particles with small size and more disruption and stabilized (Delmas et al., 2011).

Encapsulated characterizations

Encapsulated efficiency (EE). Figure 2 shows the encapsulated efficiency (EE) of encapsulated PCEP by using two different encapsulation techniques (freeze drying and spray drying) and two different homogenization methods (ultrasonication and ultraturrax). The results revealed that EE values were higher when freeze drying and ultrasonication were used than when using spray drying and ultraturrax. Under these conditions, the EE values of encapsulated PCEP were 86.85%, 83.69 and 85.47 mg catechin/kg for AA, PC

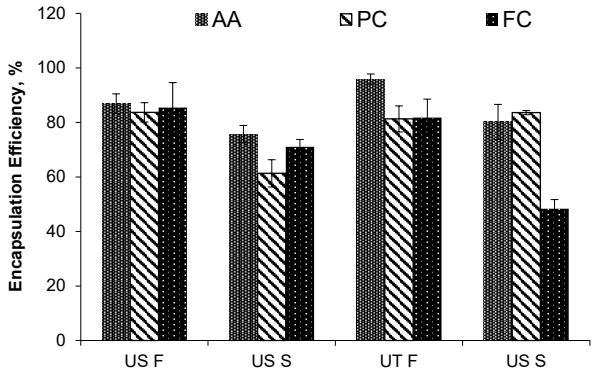

Fig. 2. EE% of encapsulated PCEP using freeze-drying (F), spray drying (S) US and homogenized by ultraturrax and ultrasonication: US F – freeze drying homogenized by ultrasonication, UT F – freeze drying homogenized by ultraturrax, US S – spray drying homogenized by ultrasonication, UT S – spray drying homogenized by ultraturrax

and FC respectively when encapsulated by freeze drying and using ultrasonication, while EE values were 80.21%, 83.58% and 48.31% mg catechin/kg for AA, TPC and TF respectively when encapsulated by spray drying and using ultraturrax. These results agree with Ramírez et al. (2015), who reported that the encapsulated efficiency was high when subjected to freeze-drying and TPC, AA, and TF are retained within the capsule matrix at a high ratio. Chen et al. (2013) and Dickinson et al. (2003) reported that spray drying forms microcapsules at the last stage of the drying process and this leads to a high incidence of entrapment of extract content and a decreasing of surface content. In contrast, freeze drying allows droplet-to-droplet interaction in the extract until the drying stage and this leads to a higher consumption of time than spray

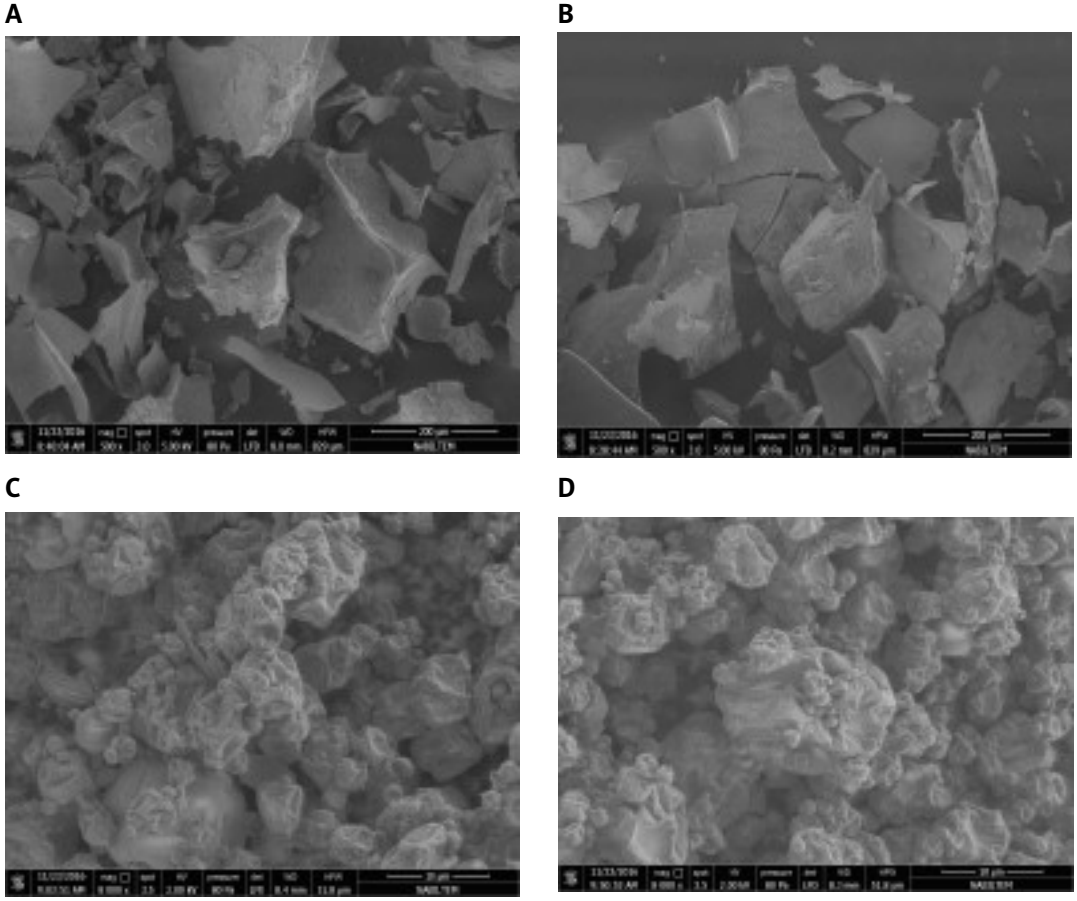

Fig. 3. SEM of encapsulated PCEP particles by freeze-drying and spray-drying and homogenized by ultrasonication and ultraturrax: A – freeze drying and ultraturrax, B – freeze drying and ultrasonication, C – spray drying and ultraturrax, D – spray drying and ultrasonication

drying, resulting in an inconsistency in the entrapment of the extract of the freeze-dried encapsulated powder which leads to a low incidence of entrapment of extract content and thereby, a high surface content.

Scanning electron microscope (SEM). The images of the particle structure for samples encapsulated by freeze-drying and spray-drying techniques are shown in Figure 3. Scanning electron microscope of encapsulated samples revealed that the freeze-dried powder seemed to have sharper edges, a broken glass-like surface, and a brittle texture which was due to a lyophilized process and a longer drying period (Khazaei et al., 2014). Sahin-Nadeem et al. (2013) mentioned that the outer topography of the spray dried particles was a spherical shape with a shallow dent of shrinkage and without cracks or pores, resulting from the protein in the wall material. The absence of pores/cracks on the particle surface is very important for preventing the inward diffusion of oxygen and hence for better protection of the encapsulated extract. Moreover, this significantly enhanced the oxidative stability of spray dried microcapsules compared to freeze dried ones, which is partly due to a lower overall surface area and a lower surface extract content.

pH and titratable acidity (TA). The changes in pH and acidity of yoghurt samples during 15 days of storage at 4°C are shown in Table 3. The pH values in yoghurt samples containing encapsulated PCEP, fresh and after 15 days, was lower than in the control sample while it decreased after 15 days during cold storage for all samples ($P < 0.05$). On the other hand, TA of the control sample was lower than yoghurt samples fortified with encapsulated PCEP when fresh and after 15 days of storage, and these values were increased during cold storage for all samples. This increase in TA indicates that microorganisms are more active in the presence of encapsulated PCEP (Kailasapathy et al., 2000).

Color analysis of yoghurt supplemented with different concentrations of encapsulated PCEP. Color plays an important role in the food choice of consumers. Table 4 shows the measurement of yoghurt color in terms of lightness (L), red-green axis (a*) and yellow-blue axis (b*). Color characteristics for yoghurt samples ranged from 88.380 ±0.168 to 75.807 ±0.040 for lightness (L*), –4.193 ±1.276 to 1.360 ±0.000 for redness (a*), and 8.600 ±1.433 to 12.203 ±1.218 for yellowness (b*). From these results, lightness (L*) values of the control sample were higher than other samples. Whiteness in plain yoghurt results from the presence of colloidal particles, such as milk fat globules and casein micelles, capable of scattering light in the visible spectrum (Fox and McSweeney, 1998). The samples fortified with encapsulated PCEP have lower lightness than the plain yoghurt due to the presence of pigments in PCEP such as anthocyanin, which agrees with Hashim et al. (2009). The a value (redness) significantly decreased for yoghurt samples fortified with encapsulated PCEP, whereas the b value (yellowness)

Table 3. pH and TA of yoghurt samples supplemented with different concentrations of encapsulated PCEP

Concentrations of encapsulated PCEP, %	Titratable acidity – TA, %			PH		
	storage period, day			storage period, day		
	fresh	7	15	fresh	7	15
Control	0.90 ±0.014^{b}	0.92 ±0.021^{b}	0.97 ±0.014^{b}	4.66 ±0.0141^{a}	4.61 ±0.014^{a}	4.59 ±0.0141^{a}
5	0.93 ±0.014^{b}	0.95 ±0.014^{b}	0.98 ±0.014^{b}	4.63 ±0.0141^{a}	4.59 ±0.021^{b}	4.55 ±0.0141^{a}
10	0.96 ±0.007^{b}	0.99 ±0.007^{b}	1.12 ±0.021^{a}	4.61 ±0.0141^{a}	4.56 ±0.014^{b}	4.51 ±0.0141^{a}
15	0.98 ±0.021^{b}	1.11 ±0.014^{a}	1.14 ±0.021^{a}	4.58 ±0.0141^{b}	4.51 ±0.014^{b}	4.46 ±0.0141^{b}
20	1.05 ±0.078^{a}	1.13 ±0.021^{a}	1.16 ±0.014^{a}	4.54 ±0.0071^{b}	4.49 ±0.021^{b}	4.43 ±0.0071^{b}

Means with different superscripts column (a, b, c) differ significantly ($P < 0.05$) from each other.

Table 4. Color parameters of fresh yoghurt samples supplemented with different concentrations of encapsulated of PCEP

Concentrations of PCEP, %	*L**	*a**	*b**
Control	88.380 ±0.168[a]	–4.193 ±1.276[c]	8.600 ±1.433[d]
5	88.503 ±0.006[a]	–3.427 ±1.241[d]	8.600 ±0.017[d]
10	85.473 ±0.202[b]	–2.850 ±0.736[c]	9.433 ±0.586[c]
15	83.923 ±0.078[c]	–1.287 ±0.771[b]	10.380 ±0.661[b]
20	79.277 ±0.126[d]	0.153 ±0.975[a]	10.200 ±1.001[a]

*L** – lightness, *a** – negative value means green and positive value means red, *b** – negative value means blue and positive value means yellow.
Means with different superscripts column (a, b, c) differ significantly ($P < 0.05$) from each other.

increased. The *a* value (redness) decreased from 4.193 ±1.276 (plain yoghurt) to 1.360 ±0.000 with the addition of up to 5% encapsulated PCEP, whereas the *b* value (yellowness) increased significantly. Increasing the amount of encapsulated PCEP in yoghurt gave it a more yellowish color, which agrees with Chung et al. (2010).

Apparent viscosity. The viscosity of plain yoghurt with encapsulated PCEP was determined with a viscometer as shown in Figure 4. The viscosity of yoghurt with added PCEP decreased significantly compared to plain yoghurt (control). The decrease in viscosity may be attributed to a lower content of casein, because PCEP encapsulated replaced a part of SMP in yoghurt.

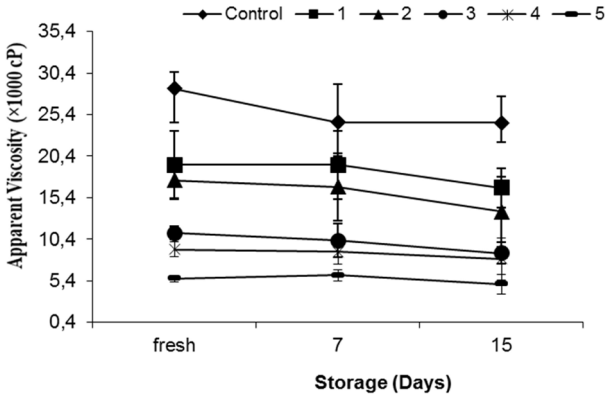

Fig. 4. Apparent viscosity of yoghurt samples supplemented with different concentrations of encapsulated PCEP

CONCLUSION

At the end of this study encapsulated apple peel extract could be a suitable phenolic compounds for adding to yoghurt. Different encapsulated techniques (freeze-drying and spray-drying) and different homogenization methods (ultrasonication and ultraturrax) were used. The encapsulated PCEP by freeze-drying and homogenized by ultrasonication was more stable in the supplemented yoghurt in comparison with the non-encapsulated PCEP with improved functionality. Encapsulated PCEP had a light effect on the physicochemical properties of the supplemented yoghurt in terms of pH, titratable acidity and viscosity. Therefore, this study suggests that PCEP microcapsules have the potential to be used as a functional food ingredient for healthy yoghurt.

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