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Characterization of peppermint (*Mentha piperita* L.) essential oil encapsulates

Murat Yilmaztekin[1*], Steva Lević[2], Ana Kalušević[2], Mustafa Cam[3], Branko Bugarski[4], Vesna Rakić[2], Vladimir Pavlović[2] and Viktor Nedović[2]

[1]Inonu University, Faculty of Engineering, Department of Food Engineering, 44280, Malatya, Turkey

[2]University of Belgrade, Faculty of Agriculture, Department of Food Technology and Biochemistry, Nemanjina 6, 11 080 Belgrade-Zemun, Serbia

[3]Erciyes University, Faculty of Engineering, Department of Food Engineering, 38039, Kayseri, Turkey

[4]University of Belgrade, Faculty of Technology and Metallurgy, Department of Chemical Engineering, Karnegijeva 4, 11 120 Belgrade, Serbia

*Corresponding author.

Murat Yilmaztekin

Inonu University, Faculty of Engineering, Department of Food Engineering, 44280, Malatya, Turkey

Phone: +90 422 377 47 84

Fax: +90 422 341 00 46

E-mail address: murat.yilmaztekin@inonu.edu.tr

Running title: Peppermint (Mentha piperita L.) essential oil encapsulates

Abstract

Aims: The aim was to choose the optimal encapsulation system and to incorporate encapsulates loaded with essential oil into ice cream as a model food product.

Methods: Ca-alginate beads were produced by electrostatic extrusion process. Gelatin/alginate coacervates were processed with coacervation. Carnauba wax microparticles were produced using melt dispersion process. Morphological properties, chemical and thermal stabilities of encapsulates were tested by SEM, FTIR spectral and thermogravimetric analysis.

Results: Alginate provided sufficient emulsion stability over 1 h. Ca-alginate showed higher encapsulation efficiency (98.4±4.3%) compared to carnauba wax (94.2±7.8%) and gelatin/alginate coacervates (13.2±1.2%). The presence of essential oil in the all three type of encapsulates confirmed with FTIR. The encapsulation process ensured controlled release and thermal stability of oil.

Conclusions: Ca-alginate matrix as the most suitable for peppermint essential oil encapsulation. The sensory analysis showed that ice cream incorporating encapsulates is promising system for consumption of health beneficial peppermint essential oil.

Keywords: *Mentha piperita* L. essential oil, encapsulation, ice cream, sensory evaluation

1. Introduction

Essential oils have a huge historical and practical significance for human civilization, primarily as therapeutics for treatment of various diseases, as well as ingredients in cosmetics and food products (Burt, 2004). The peppermint is rich source of valuable natural compounds with antimicrobial, antioxidant and antitumor activity (McKay and Blumberg, 2006). Peppermint essential oil is traditionally produced and widely used in medicine, massage and for personal hygiene (Ody, 1993). Also, peppermint essential oils could be used as source of monoterpenes that are suitable for treatment of some cancers types (Crowell, 1997).

Due to exposure to unfavorable environmental conditions such as high temperatures or oxygen, the essential oils may lost their valuable ingredients and consequently their health benefits. Volatile and chemically unstable components of essential oils could be additionally protected by the process commonly known as encapsulation. Encapsulation could be defined as entrapping of active compound (core) into structure of another material (shell) (Gibbs et al., 1999). Numerous encapsulation techniques such as spray drying, fluid bed coating, spray-cooling, melt injection, coacervation and encapsulation in polymer microsphere have been developed for encapsulation of food active compounds (Zuidam & Shimoni, 2010). One of the promising encapsulation methods is entrapping of active compound inside polymer matrix. Among natural polymers, alginate has been intensively used for encapsulation of food additive (Manojlović et al., 2008) and essential oils (Chang & Dobashi, 2003; Lai et al., 2007). Further, the size of alginate based encapsulates could be controlled using electrostatic droplet generation, enabling production of spherical calcium alginate particles suitable for various biotechnological applications (Nedović et al., 2001; Manojlović et al., 2008; Lević et al., 2013).

Another promising encapsulation technique for protection and controlled delivery of essential oils is coacervation. Encapsulates produced by coacervation (or simple coacervates)

could be defined as encapsulation system produced by liquid-liquid phase separation of polymer(s) aqueous solution. By careful adjustment of temperature and pH, it is possible to create polymer layer around dispersed oil droplets. After formation, coacervates are separated from solution and could be further processed by crosslinking of shell material or processing coacervates by drying (Zuidam & Shimoni, 2010). Coacervation has been applied for encapsulation of aromatic compounds using different natural shell materials. Jun-xia et al. (2011) showed that coacervation between soybean protein isolate and gum arabic can be used for encapsulation of sweet orange oil into spherical shaped particles. Also, heat resistance of essential oil could be improved using coacervates based on gelatin/gum arabic (Lv et al., 2014).

Encapsulation of highly hydrophobic active materials such as essential oils could be achieved by applying hydrophobic shell material. This approach has been considered for encapsulation of pharmaceutical compounds inside hydrophobic natural compounds such as waxes. Usually, preparation of wax particles are based on dispersion of molten mixture wax/active compound in hot water followed with solidification by cooling and fast solidification of lipid carrier enables efficient entrapment of hydrophobic compounds inside wax microparticles (Milanović et al., 2010).

The aim of this study was to investigate the possible solutions for essential oils encapsulation. Peppermint essential oil was used in this study as model core compound, while Ca-alginate, gelatin/alginate and carnauba wax were carriers for encapsulation. In order to eliminate water and provide better storage performance on encapsulates, the samples were freeze dried..The morphology, chemical composition and thermal properties of encapsulates were studied in order to define optimal system for peppermint essential oil encapsulation. Considering potential health benefits of using peppermint essential oil in food industry, production of encapsulates were optimized in that respect that the final encapsulates could be

applied in various food products. Carnauba wax beads loading peppermint essential oil were chosen as optimal encapsulation system and were incorporated into ice cream as a model food product. Sensory analysis was carried out on ice cream samples enriched with carnauba wax beads containing peppermint essential oil.

2. Materials and methods

2.1. Materials

Peppermint essential oil (Arifoglu brand) was supplied from a local market (Kayseri, Turkey). The chemical composition of essential oil determined by GC-MS was given in Table 1. The alginate (ALGOGEL^{TM}3001, Cargill, Minnetonka, US) was generously donated by PALCO (Šabac, Serbia). Bovine gelatin (food grade) was supplied from domestic market in Belgrade (Serbia). Carnauba wax was supplied from Carl Roth GmbH (Karlsruhe, Germany). Sodium citrate and dichloromethane (GC grade) were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical reagent grade and used without any further purification.

2.2. Encapsulation of peppermint essential oil in Ca-alginate beads

The production of Ca-alginate beads was realized in two phases. First phase was preparation of liquid systems-emulsions that are used for beads production. Prior to preparation of emulsion, Na-alginate was dissolved in distilled water (concentration of 0.02 g/mL). Further, different ratios of peppermint essential oil to Na-alginate (10 and 20%, w/w oil in alginate solution) were mixed in order to produce stable emulsions. The emulsions were prepared using mechanical stirrer Ultra-Turrax® T25 (Janke and Kunkel Ika-Labortechnik, Germany) at 10000 rpm for 2 minutes. Emulsions prepared in this way remain stable over the 1 h, which is period of time enough for emulsion extrusion and beads formation. The addition

of emulsifiers was not necessary since alginate provided sufficient emulsion stability, which is in accordance with previously reported results (Lević et al., 2015).

In the second phase, the Ca-alginate beads with encapsulated peppermint essential oil were produced by procedure described previously by Nedović et al. (2001). Droplets were formed by extrusion of the sodium alginate/essential oil emulsion through a blunt stainless steel needle using a syringe pump (Pump 11, Harvard Apparatus, US). The needle was grounded, while the collecting solution ($CaCl_2$ in water, 0.015 g/mL) was positively charged. Beads production was carried out by applying electrostatic potentials of 8 kV. Electrostatic encapsulation unit (VAR V1, Nisco Engineering Inc., Zurich, Switzerland) was used in this work for beads production under electrostatic potential. The distance between the needle tip (id: 0.7 mm) and the collecting solution was 2.5 cm, while the flow rate of the liquid was 70 mL/h. After formation of the beads, they were left in hardening solution without stirring for 60 min in order to assure finishing of the gelling process. The formed alginate beads were removed from the $CaCl_2$ solution by filtration and washed with distillated water. After gel formation period, the beads were frozen at -20 °C overnight before freeze drying, which was carried out at -50 °C at a pressure of 1.1 Pa for 24 h in freeze drier (GAMMA Martin Christ, GmbH, Osterode am Harz, Germany).

2.3. Encapsulation of peppermint essential oil in gelatin/alginate coacervates

Formation of coacervates loading peppermint essential oil was performed according to procedure described by Siow and Ong (2013), with modifications. The main modifications were usage of Na-alginate as carrier material and citric acid for pH control instead of gum arabic and acetic acid, respectively. Gelatin (concentration of 0.02 g/mL) and Na-alginate solutions (concentration of 0.02 g/mL) were prepared by dissolving these compounds in distilled water. 25 mL of each gelatin and Na-alginate solutions were mixed with 1, 2 and 4 g

of essential oil at 45 °C by a mechanical stirrer Ultra-Turrax® T25 (Janke and Kunkel Ika-Labortechnik, Germany), respectively. A solution of 10% (w/v) citric acid was added to mixture to adjust pH to 2-3. Such relatively low pH was applied in order to ensure fast coacervates formation and to minimize the loss of essential oil during preparation steps. The mixture was cooled down immediately to 10 °C in an ice bath. Further, the coacervates were collected by filtration, washed with distillated water and freeze dried as described above.

2.4. Encapsulation of peppermint essential oil in carnauba wax

Encapsulation of peppermint essential oil in carnauba wax was realized by melt dispersion technique (Milanović et al., 2010). Carnauba wax was melted in distilled water at 95 °C in a water bath. Peppermint oil was added to the dispersion of molten wax in water while stirring rigorously (10000 rpm for 2 min) by a mechanical stirrer Ultra-Turrax® T25 (Janke and Kunkel Ika-Labortechnik, Germany). Different weight ratios of essential oil to wax (10, 20, 30 and 50 %, w/w) were tested. The solidification of the molten wax droplets with oil were performed by adding 150mL of cold water (~5 °C) to the resulting dispersion. After addition of cold water, dispersion was cooled down and solid particles were formed. Further, the particles were collected by filtration, washed with distilled water and freeze dried. All encapsulates were then stored at +4 °C in closed falcon tubes for further analyses.

2.5. Analysis of the bead dimensions and shape

Dimension and shape of wet and dried Ca-alginate beads as well as carnauba wax particles were evaluated by binocular microscope Leica XTL-3 400D (Leica, Germany), equipped with camera (DC 300, Leica, Germany) and software for measuring (IM 1000, Leica, Germany). For each measurement, a sample of 30 beads was taken and the diameters of the beads were

measured. The diameter for each particle was calculated as average of the horizontal and perpendicular dimensions (Lević et al., 2015).

2.6. Scanning electron microscopy (SEM)

Morphological investigations of the samples surface have been carried out by JEOL JSM-6390LV scanning electron microscope. Prior to analysis, samples were sputter-coated with gold during 100 seconds under 30mA ion current on BALTEC SCD 005 sputter coater (Lević et al., 2015).

2.7. Determination of encapsulation efficiency

Encapsulation (*EE*) efficiency of encapsulates were calculated by the following equation as the percentage of the total amount of encapsulated essential oil (m_E) in the total amount of initial input of essential oil (m_I) (Lević et al., 2015; Siow and Ong, 2013):

$$EE(\%) = (m_E/m_I) * 100 \quad (1)$$

Quantification of essential oil content in encapsulates was performed after extraction with dichloromethane. Briefly, 0.5 g of lyophilised alginate encapsulate was dissolved in 40 mL of sodium citrate water solution (1.5%, w/v) in a sealed glass bottle under vigorous mixing using a vortex mixer. When encapsulates are dissolved, 5 ml of dichloromethane was added and essential oil was extracted for 15-20 min with intermittent mixing. For carnauba wax and coacervates based on gelatin, 0.5 g of encapsulate was extracted three times with 10 mL portions of dichloromethane. After extraction organic phase was separated and subjected to GC-MS analysis.

2.8. GC-MS analysis of peppermint essential oil

Gas chromatography-mass spectrometry (GC-MS) analysis was performed in order to determine the composition of peppermint essential oil and to measure the concentration of the most represented compound, menthol, in dichloromethane extracts of encapsulates which is used for calculation of encapsulation efficiencies. The analyses were carried out on a Shimadzu QP 2010 Plus (Shimadzu, Kyoto, Japan) GC equipped with an AOC-20i/20s autosampler and a MS-QP 2010 series mass-selective detector. The conditions of GC-MS were adapted from Yilmaztekin (2014). Sample (1 μL) was injected into the injector with a split ratio of 1:50. Volatile compounds were separated with a TRB-Wax (Teknokroma, Barcelona, Spain) fused silica capillary column (60 m × 0.25 mm i.d. and 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The column was maintained at 40 °C for 5 min after injection, and then programmed at 3 °C/min to 240 °C, which was maintained for 15 min. The total run time, including oven cooling, was 86 min. Injector, transfer line, and ion-source temperatures were maintained at 250 °C. Mass spectrums were acquired in electron-impact (EI) mode; the ionization voltage was 70 eV; the mass range was 35-450 m/z; scanning rate was 1 scan/s. A mixture of n-alkanes (C_8-C_{28}) was injected under the above conditions to calculate the linear retention indexes (as Kovats' indice, I) of each compound. The identification was made by comparison of the obtained mass spectrums of the relevant chromatographic peaks with spectrums of the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley libraries. In addition, tentative identification was carried out by comparing the experimental retention indices with the theoretical ones obtained from the NIST Standard Reference Database (NIST 2013). Quantitative analysis of essential oil components (expressed as area percentage) was carried out by peak area normalization measurement. Menthol was identified by comparing

the retention times and spectral data with those of the appropriate standard. The amount of essential oil in beads was calculated by calibration curve obtained from dichloromethane extracts of known amounts of essential oil.

2.9. FTIR spectral analysis

FTIR spectra of the freeze dried encapsulates was studied using IRAffinity-1 (SHIMADZU) FTIR spectrometer. Spectra were collected using KBr pellets (1 mg of sample in 200 mg of KBr) in the spectral range 4000-600 cm^{-1}. Spectra were recorded as average curves from 20 acquisitions, with the resolution of 4 cm^{-1} (Lević et al., 2013). FTIR spectra of free peppermint essential oil (2 μL) were recorded using two blank KBr tablets that were used to create a thin liquid sample film.

2.10. Thermal analysis of the samples

Thermal analyses of freeze dried samples were carried out in a Setaram's system TG/DSC111. The measurements were realized under dynamic oxygen of a flow rate of 30 mL/min (pressure 1 atm) using a heating rate of 10 °C/min from 50 to 300 °C.

2.11. Production of ice cream incorporated with carnauba wax oil beads

Due to small size of the particles and relatively satisfactory encapsulation efficiency, carnauba wax based encapsulates were chosen for incorporation into ice cream. Although Ca-alginate capsules exhibited better encapsulation efficiency (see below), their size could negatively affect ice cream textural properties and overall sensorial acceptance. Four types of ice cream were produced with the following combinations according to Cam et al. (2013): Control ice cream (IC1), enriched with peppermint oils at 0.1% (w/w) (IC2), 0.2% (w/w) (IC3) and 0.3% (w/w) (IC4). The amount of peppermint oil in the samples was based on dry

matter content. As an example, ice cream ingredients for IC2 were mixed in the following combinations: A glass beaker containing 180 mL of skim milk was place on a thermostatically controlled mechanical stirrer. Ingredients were added into the beaker at 50 °C with regular stirring at 1000 rpm. Cream (40 g), skim milk powder (25 g), sucrose (32 g), mono-di glyceride mix (0.5 g), sahlep as stabilizer (0.5 g) and encapsulated peppermint oil (0.1 g). The mixture was pasteurized at 80 °C for 10 min followed by cooled to +4 °C. After keeping the mixture at +4 °C for 24 h the aged mixture was whipped at 0 °C for 30 min with a laboratory type ice cream machine (Gelato, Bologna, Italy). The resulting ice cream was hardened at -18 °C for 24 h in a deep freezer. All other formulations (IC1, IC3, and IC4) were prepared in the same way by changing the peppermint oil content but keeping the amount of other ingredient's constant.

2.12. Quantitative descriptive sensory analysis of ice cream incorporated with carnauba wax oil beads

Six nonsmoking panelists had previous experience in testing ice cream (3 female and 3 male, ages between 26 and 39) from Department of Food Engineering in Erciyes University were selected for the evaluation of the sensory attributes of the ice creams. The panelists were trained until they fully understood the sensory terms of ice creams and showed consistent replicates. Reference sample preparation and administration of the panel were conducted according to relevant literature (Hightower and Edgar, 2009; Soukoulis and Tzia 2010; Cam et al., 2013).

Flavor descriptors of ice creams were selected as: palatable, sweet, sour, astringent, and peppermint flavor. A 10-point scale was used for evaluations, where 0 for nonexistence, 5 for imperceptible, 10 for strong presence of related attributes. The definitions of the descriptors and sample references are given in Table 2.

2.13. Statistical analysis

All chemical and instrumental analyses were done minimum in triplicate. Ice cream formulations were prepared minimum in duplicate. Analysis of variance was performed to determine significant differences between the shell materials for bead sizes and encapsulation efficiencies at $P \leq 0.05$ significant level using the SPSS 17.0.1 statistical package for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

3. Results and discussion

3.1. Bead size, encapsulation loading and encapsulation efficiency of peppermint essential oil encapsulates

Ca-alginate, gelatin/alginate coacervates and carnauba wax microparticles were used as carriers for encapsulation of peppermint essential oil. Table 3 shows the average size and encapsulation efficiencies of peppermint essential oil encapsulates.

As can be seen, Ca-alginate as carrier material showed higher encapsulation efficiency (98.4±4.3%), followed by carnauba wax microparticle (94.2±7.8%), while gelatin/alginate coacervates exhibited the lowest encapsulation efficiency (13.2±1.2%). These results are in accordance with the results from other authors who also reported high encapsulation efficiency of Ca-alginate and carnauba wax as carriers for encapsulation of hydrophobic compounds (Lević et al., 2015; Milanović et al., 2010). Evidently, gelatin/alginate coacervates are far less suitable for encapsulation of peppermint essential oil regarding encapsulation efficiency. This is contrast to results reported in other studies that usually showed high encapsulation efficiency of coacervates loading essential oils and aromatic compounds (Manaf et al., 2018). Low encapsulation efficiency of the gelatin/alginate coacervates prepared in this study could be explained by repulsive forces between hydrophilic

carriers and essential oil as well as complex structure of peppermint essential oil. Also, McClements and Rao (2011) pointed out that formation of stable emulsion of essential oil is difficult process since smaller oil droplets tend to coalescence into larger droplets, leading to emulsion breakdown. Furthermore, additional processing of coacervates such as drying may cause further loss of essential oil. This was observed by Shinde & Nagarsenker (2011) for the gelatin/alginate coacervates loading eugenol. The same authors reported similar encapsulation efficiency as we observed in this study, and as the main reasons for low retention of eugenol they suggested the dehydration of coacervates which caused structural changes of particles causing eugenol leakage.

The particles of Ca-alginate are larger compared to wax particles, while gelatin/alginate coacervates after drying were in the form of aggregated mass that could not be studied for their size.

3.2. Morphological properties of peppermint essential oil encapsulates

Morphological properties of encapsulates loading peppermint essential oil were studied by scanning electron microscopy and results are presented in Figure 1. After encapsulation and gelling process, wet Ca-alginate beads exhibited spherical shape. However, during the freeze drying, the water were removed from the beads and the alginate matrix shrank significantly (Figure 1a, b).

According to Lević et al. (2013) addition of solid flavor into alginate matrix provide better preservation of beads shape and sphericity during drying process. It seems that solid filers (i.e. active compounds) are more suitable for shape stabilization of encapsulate compared to liquid active compounds. In some cases, preservation spherical shape of encapsulate is favorable (e.g. positive visual effects on consumers). Introduction of additional filer in formulation could be solutions for problem of shape maintenance during drying of alginate

based spherical beads. Chan et al. (2011) proposed using of starch as filler substance for shape preservation of lyophilized Ca-alginate beads loading cells.

As we pointed out above, freeze dried coacervates (Figure 1c,d) show significant changes of morphological properties during drying process. Namely, after encapsulation, coacervates remained free flowing in working solutions and were easily collected. During drying process, the structure of coacervates changes toward compact-flakes like structure. On the other hand, microparticles based on carnauba wax (Figure 1e) show more regular spherical shape compared to other formulations, although their sizes were not uniform. According to Milanović et al. (2010) the size of carnauba wax microparticles produced by melting dispersion technique could be controlled by variation of process conditions. Compared to other formulations (i.e. Ca-alginate and gelatin/alginate coacervates) carnauba wax particles exhibit significant surface porosity (Figure 1f). Porosity of wax microparticles is most probably consequence of essential oil addition that causes changes of particles structure during encapsulation. Irregular surface of carnauba wax particles was also observed by Milanović et al. (2010).

3.3. The results of FTIR analysis

The FTIR spectra of the free peppermint essential oil and encapsulate loading oil are presented in Figure 2. In the spectrum of free essential oil, the strong band at 3470 cm^{-1} is assigned to the vibrations of the -OH groups, while bands at 2924 cm^{-1} and 2854 cm^{-1} appeared due to the presence of the $-CH_3$ and $-CH_2$ groups. The bands in the spectral range 1700-1750 cm^{-1} are most probably related to C=O vibrations. Other band such as those at 1460 cm^{-1} and 1377 cm^{-1} originate from various organic compounds, which presence in the peppermint essential oil was verified by GC/MS analysis (see Table 1). Prakash and Yunus (2013) showed that vibrations assigned to menthol and menthone (in the spectral range 2849-

2954 cm^{-1}), dominate in the FTIR spectrum of essential oil produced by hydro-distillation of *Mentha arvensis*. We also observed similar prominent bands in the same spectral region of both free and encapsulated peppermint essential oil. Also, the results of GC/MS analysis of *Mentha piperita* L. essential oil conducted in this study suggest that these two compounds are present in the high concentration.

The results of FTIR analysis show the presence of essential oil in the all three type of encapsulates produced in this study. Moreover, the spectra of carriers (i.e. Ca-alginate, gelatin/alginate coacervates and carnauba wax) are generally overlapped by spectrum of essential oil. Some relatively small variations in the spectra of encapsulate such as differences in bands shapes or intenisties might be due to carrier/oil bands overlapping. These findings clearly show that essential oil and carrier materials most probably made a simple mixture in the encapsulates, since there is no strong evidence of chemical interactions between peppermint essential oil compounds and carrier materials.

3.4. The results of thermogravimetric analysis

Thermal stability of free and encapsulated peppermint essential oil was investigated by thermogravimetry and results are presented in Figure 3. Free peppermint essential oil shows first mass loss in the temperature region 90-200°C (~50% of mass loss). In the second temperature region, between 200°C and 300°C up to ~60% of essential oil evaporates. The two steps in the TGA thermogram are most probably result of different boiling points of essential oil compounds. Also, it could be expected that some essential oil compounds are thermal decomposed to the new compounds. According to Neuenschwander et al. (2010), α-pinene autoxidation under elevated temperature leads to formation of different types of peroxyl radicals. Further, peroxyl radicals' reaction leads to production of hydroperoxide or epoxide and alkoxyl radicals.

In the case of Ca-alginate/essential oil, thermogram shows that up to 150°C the mass loss of beads is <10%. Further, mass loss is most probably connected with oil compounds evaporation/thermal decomposition as well as Ca-alginate thermal decomposition. The release of encapsulated peppermint essential oil and thermal decomposition of Ca-alginate matrix most probably occurred in the close temperature regions. According to Lević et al. (2013), the main mass losses of empty Ca-alginate beads correspond with two temperature regions: (i) 50-150°C and (ii) 150-300°C. Laurienzo et al. (2005) pointed out that mass loss in the first temperature region is connected with loss of water from alginate matrix. The second temperature region corresponds with mass loss of encapsulated oil (and most probably with oil thermal degradation) and with thermal decomposition of Ca-alginate. However, thermal degradation of alginate is complex process including several steps.

The TGA thermogram of encapsulated peppermint essential oil in gelatin/alginate coacervates shows two main mass losses. Up to 175°C the mass loss is 10-15% and most probably is connected with water loss from matrix material as well as essential oil release. In the temperature region from 50°C to 175°C, the mass loss of coacervates with encapsulated oil is very similar to those based on Ca-alginate. However, at the end of analysis (at 300°C), the coacervates show higher mass loss (~ 70% mass loss) than systems based on Ca-alginate (i.e. ~50% mass loss).

Coacervates showed lower encapsulation efficiency compared to encapsulate produced by other encapsulation methods used in this study (see Table 2). As result, the kinetic of mass loss of coacervates at high temperatures is most probably affected by low essential oil content. Also, the reason for difference in the thermal properties of alginate beads and coacervates may be the fact that Ca-alginate beads are produced by gelling of sodium alginate using Ca^{2+} ions as crosslinker. Thus, Ca-alginate is most probably more thermal stable matrix compared to gelatin/alginate coacervates that are not crosslinked. Devi et al. (2012) suggested

that additional crosslinking using glutaraldehyde as crosslinker provides better thermal properties of gelatin/alginate microcapsules produced by complex coacervation. However, the usage of glutaraldehyde is limited in food industry. This problem could be for example solved by using enzymatic crosslinking of gelatin (Prata et al., 2008).

The thermogram of wax based encapsulates exhibit two mass losses: up to ~250°C (max. at 130ºC, probably wax decomposition and oil release); the second mass loss occurs above 250°C and it is most probably connected with decomposition of carnauba wax. The release of essential oil is more pronounced at higher temperatures (above 80-85ºC) as melting point of carnauba wax corresponding to this temperature range. Milanović et al. (2010) noticed that thermal processes above 250°C are results of carnauba wax transformation under the high temperatures. Since wax particles under elevated temperature (i.e. 80-85ºC, carnauba wax melting point) are melted, such wax-based encapsulates are more suitable for applications in the low temperature food processes. We've selected ice cream as model food for incorporation and sensory analysis of peppermint oil encapsulated in the carnauba wax particles. Based on thermal analysis, the alginate and coacervates encapsulates of peppermint essential oil are more suitable for applications in the high temperature food processes (e.g. baker's products). Also, smaller in size, carnauba microparticles are more suitable regarding ice cream textural properties.

Thermal analyses showed that encapsulated essential oil was released at elevated temperatures in the broader temperature regions and in more controllable manner compared to free peppermint essential oil. These results suggest that encapsulated peppermint essential oil is most probably more suitable for thermal food processes, enabling protection and controlled release of aromatic compound into product. In this study, we tested applications of carnauba wax microparticles loading peppermint essential oil in ice cream. However, further studies are

necessary in order to evaluate applications of encapsulated peppermint essential oil in other thermal processed food products.

3.5. Quantitative descriptive sensory evaluation of ice cream incorporated with carnauba wax oil beads

Ice cream samples, control and enriched with peppermint oil, showed no statistically significant differences in terms of textural properties and color in quantitative descriptive sensory analysis. There were no differences among 4 ice cream types in terms of textural properties e.g. hardness, coarseness, gumminess, iciness, wateriness, and creaminess. The same situation was observed for color as well. Therefore, texture and appearance of ice cream samples were not included here.

The increase in the amount of peppermint oil in ice creams imparted peppermint oil flavor to the samples (Figure 4). Sourness and astringency of ice creams were slightly perceived by the panelists, however, the degree of sourness and astringency were lower than the value of 4. Refreshing peppermint oil flavor were noted aftertaste by the panelists. Panelists rated the overall acceptability of all peppermint oil enriched ice creams higher than the value of 7. Therefore, peppermint oil enrichment might be a suitable option to produce new ice cream types. We have also tested the possibility of peppermint oil enrichment higher than 0.3% (w/w) in ice cream, however, oral burn and dramatic decrease in the overall acceptability were noted by panelists.

4. Conclusions

Ca-alginate encapsulates showed higher encapsulation efficiencies compared to other shell materials. On the other hand, after the drying process carnauba wax microparticles were more regular spherical in shape than the other formulations. The essential oil and carrier materials

formed simple mixtures inside the encapsulates, without strong chemical interactions. The results of thermal analysis showed that encapsulation of peppermint essential oil ensured controlled release of oil in the broader temperature regions compared to free oil. According to sensory analysis, incorporation peppermint oil into ice cream up to 0.3% (w/w) might be a suitable option to add functional properties of peppermint into ice cream without damaging textural properties. Since tested encapsulation systems are different in many aspects, our goal was to establish different procedures for essential oils encapsulation and to open the possibility for their application in encapsulated forms in food products. Regarding encapsulation efficiency, Ca-alginate is the most promising system for peppermint essential oil encapsulation. On the other hand, carnauba wax microparticles are suitable in the applications were small particles are required due to food textural properties (i.e. here tested ice cream with added carnauba wax microparticles loading peppermint essential oil). Coacervates showed the lowest encapsulation efficiency. However, due to its simple preparation procedure, encapsulation of essential oils by coacervation could be improved by additional steps like a crosslinking of carrier material in order to increase encapsulation efficiency.

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The Authors declare that there is no conflict of interest.

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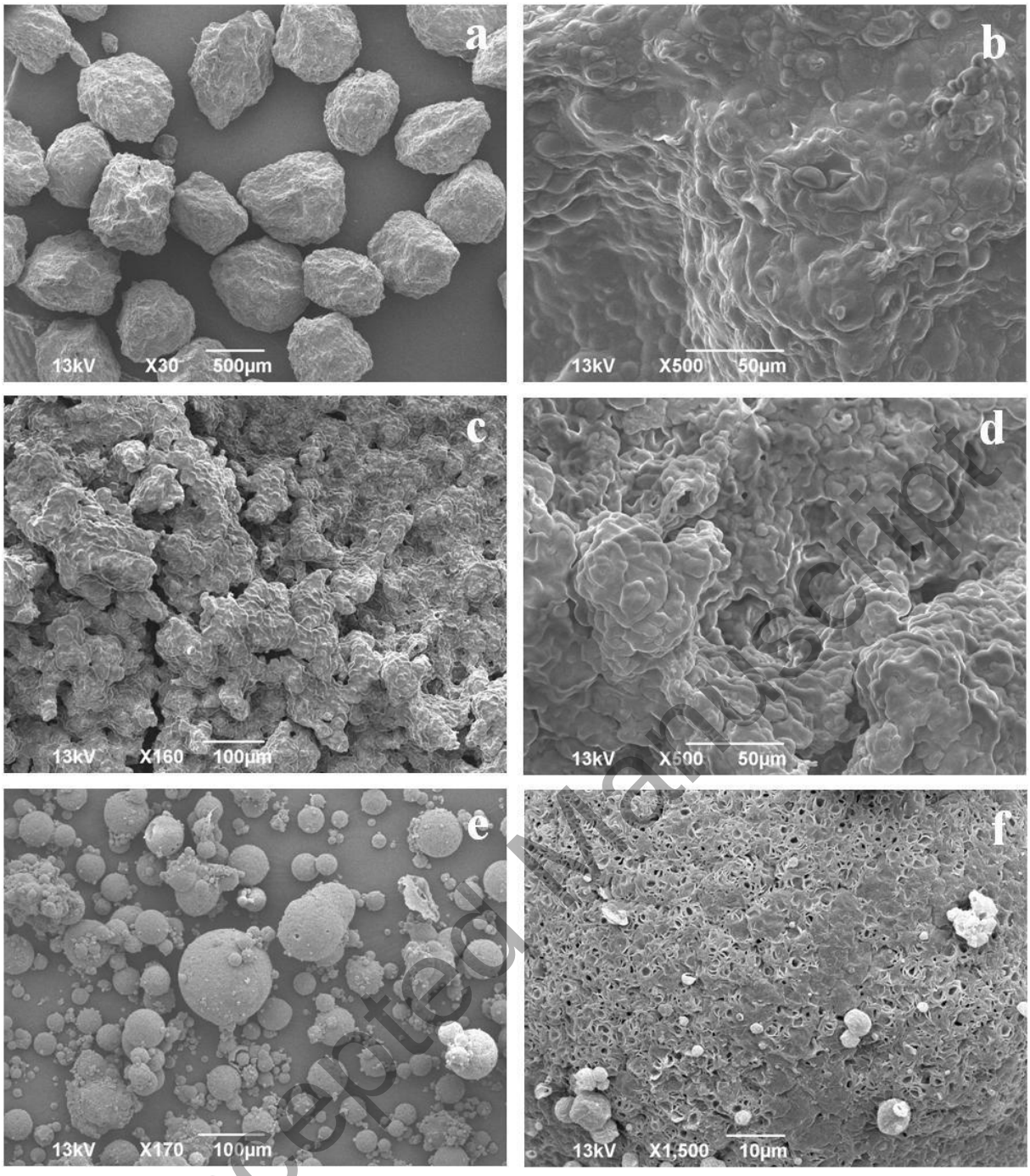
Figure captions

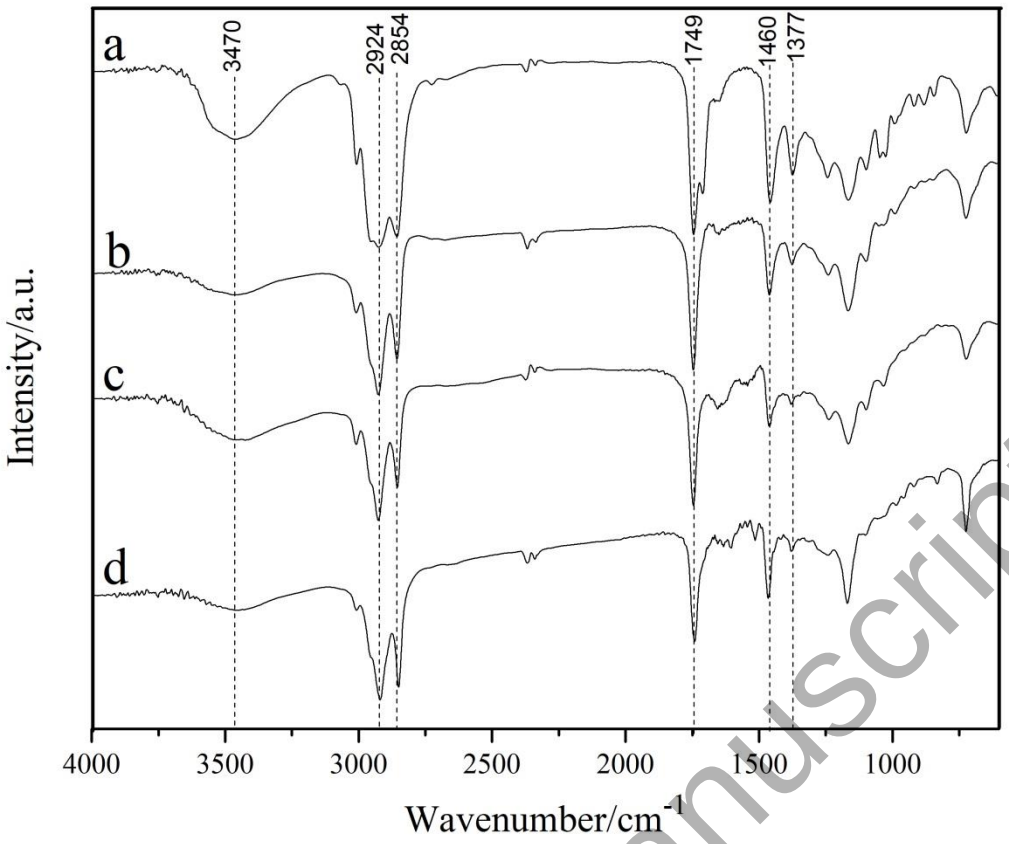
Fig. 1. SEM images: A2 (a-low magnification; b-high magnification), C4 (c-low magnification; d-high magnification), CW4 (e-low magnification; f-high magnification).

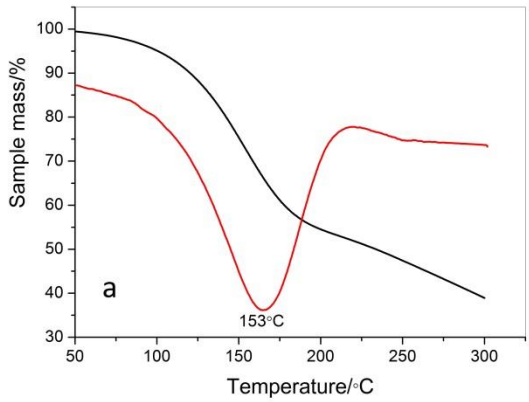
Fig. 2. FTIR spectrum of the free peppermint essential oil (a), A2 (b), C4 (c) and CW4 (d).

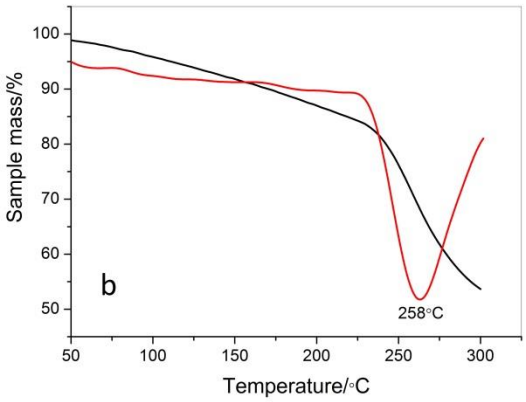
Fig. 3. Thermogravimetric analyses of essential oil (a), A2 (b), C4 (c) and CW4 (d). Black line-thermogravimetry curve; red line-differential thermal gravimetry curve.

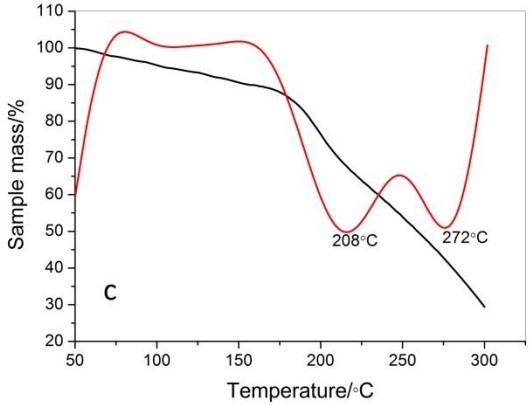
Fig. 4. Sensory scores of ice cream samples in a radar plot (IC1: Control ice cream, IC2, IC3, and IC4: Ice cream enriched with peppermint oil at 0.1, 0.2 and 0.3%, w/w, respectively).

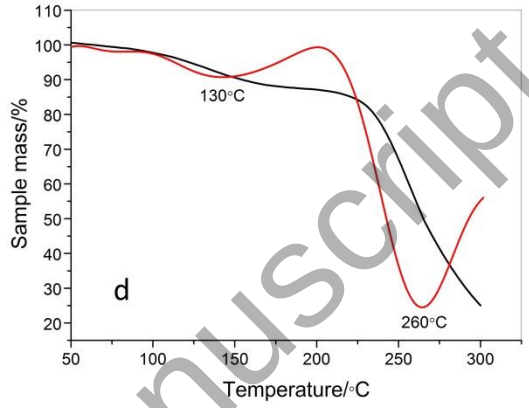












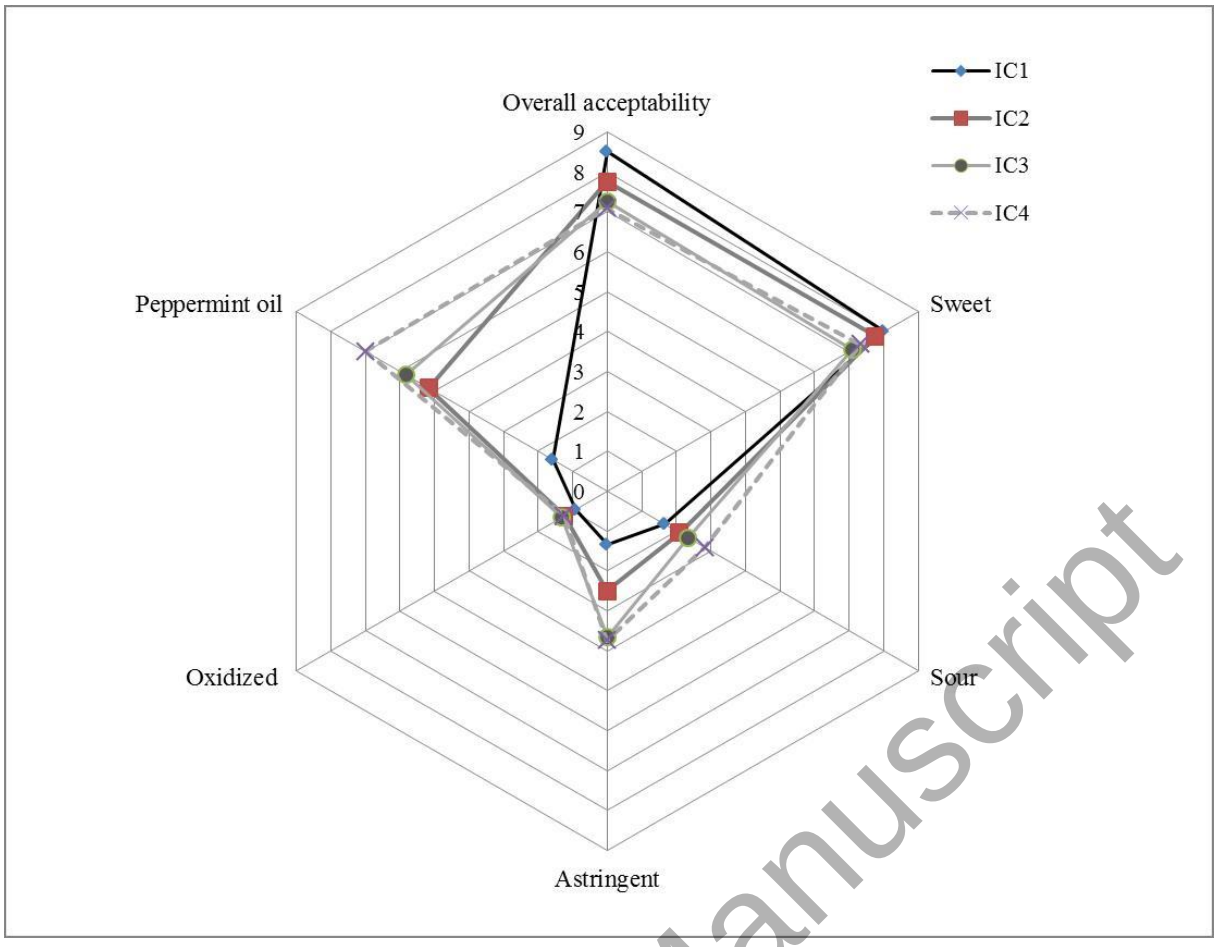


Table 1. Chemical composition of peppermint essential oil

Compound	%
α-pinene	7,55
camphene	0,23
β-pinene	8,56
sabinene	0,24
β-myrcene	0,76
limonene	9,50
1,8-cineole	0,88
β-cymene	0,27
3-octanol	0,64
p-menthone	14,89
β-bourbonene	0,83
linalool	0,37
1-octanol	0,51
menthyl acetate	8,54
isopulegol	4,30
longifolene	0,19
α-ylangene	0,39
neomenthol	6,98
β-caryophyllen	1,31
neoisomenthol	1,24
menthol	20,31
pulegone	1,83
lavandulol	0,61
α-terpineol	6,09
piperitone	2,98
total	100

Table 2. Descriptive terms, definition, and reference material used for the training of the panelists[a]

Sensory attribute	Definition	Reference material
Overall acceptability	Ice cream has no defects. Sensorial properties are well balanced.	A commercial ice cream (Algida Maraş Usulü, Istanbul, Turkey)
Sweet	The intensity of flavor caused by sucrose is clearly perceivable	2.0% (w/v) sucrose solution
Sour	It is characterized by a refreshing and fruity flavor.	A commercial sorbet (Algida, Istanbul, Turkey).
Astringent	Astringency sensation is dry and gives a puckery feeling in the mouth.	0.5% (w/v) punicalagin solution.
Oxidized	Off-flavor is characterized by acardboard, papery and tallow flavor and smell.	Cream was oxidized at 105 °C for 2 h and an ice cream mix was prepared with oxidized cream.
Overall peppermint flavor	Sweet, green, earty, sharp, mentholic aromatics associated with peppermint oil.	Peppermint oil (Arifoğlu Baharat, Istanbul, Turkey)

[a]Sensory attributes, definitions, and reference samples were based on the literatures (Hightower and Edgar, 2009; Soukoulis and Tzia, 2010, Çam et al., 2013)

Table 3. Average bead size and encapsulation efficiency of peppermint essential oil encapsulates

Preparation	Bead size (μm)	Encapsulation efficiency (%)
Calcium alginate beads		
A1	693.5 ± 133.6a	98.4 ± 4.3a
A2	827.2 ± 121.5a	96.5 ± 5.5a
Gelatin and alginate coecervates		
C1	-	13.2 ± 1.2c
C2	-	11.8 ± 1.0c
C4	-	10.5 ± 0.8c
Carnauba wax beads		
CW1	27.6 ± 15.4b	66.8 ± 4.7b
CW2	37.1 ± 18.7b	72.9 ± 5.2b
CW3	21.7 ± 10.9b	69.6 ± 5.8b
CW4	30.4 ± 14.5b	94.2 ± 7.8a

A1: 2 g oil + 18 g sodium alginate, A2: 4 g oil + 16 g sodium alginate, C1: 1 g oil + 50 g polymer, C2: 2 g oil + 50 g polymer, C4: 4 g oil + 50 g polymer, CW1: 1 g oil + 9 g carnauba wax, CW2: 2 g oil + 8 g carnauba wax, CW3: 3 g oil + 7 g carnauba wax, CW4: 5 g oil + 5 g carnauba wax. In the same column, means with different letters significantly differed ($P < 0.05$).