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ORIGINAL PAPER

Ready to drink iced teas from microencapsulated spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.) extracts: physicochemical, bioactive and sensory characterization

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Abstract

Spearmint (*Mentha spicata L.*) and peppermint (*Mentha piperita* L.) have important biological activities. They are commonly consumed as herbal teas. This study was designed to produce ready to drink iced teas rich in bioactive compounds by using microencapsulated spearmint and peppermint extracts. Pressurized hot water extracts of spearmint and peppermint were processed into microencapsulated powders using spray drying. Iced teas were produced by using the microencapsulated extracts, sucrose and citric acid. In addition, the effects of the use of microencapsulated lemon extract (MLE) instead of citric acid were studied to evaluate the potential application of MLE on iced tea production. Total phenolic content (TPC) of iced teas ranged between 27.37 and 46.31 mg gallic acid equivalents/100 mL. Antioxidant capacities (as EC_{50} and Trolox equivalent) of iced teas were between 389–481 mL/g DPPH and 55.33–80.88 mg Trolox equivalents/100 mL. The main phenolic compound was rosmarinic acid in spearmint iced teas, whereas eriocitrin was the main phenolic in peppermint iced teas. MLE usage increased TPCs, antioxidant activities and eriocitrin contents of iced teas. HPLC analysis showed hydrolysis of sucrose to invert sugar (fructose and glucose) during the production of the iced teas. Carvone amount (0.30–0.32 mg/100 mL) in spearmint iced teas and sum of menthol, menthone and methyl acetate (6.51–6.75 mg/100 mL) in peppermint iced teas were measured by GC–FID. The sensory results showed that no significant differences were between iced teas with citric acid and iced teas with MLE. Peppermint iced teas were characterized by nasal and oral cooling sensations.

Keywords Ready to drink iced tea · Spearmint · Peppermint · Microencapsulation · Enrichment

Introduction

Spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.) are notable species of genus *Mentha* in the *Lamiaceae* family [1]. Spearmint is mostly used as a seasoning in world cuisine. Carvone, a minty and sweetish odor, is the main compound (60-70%) of spearmint essential oils [2]. Furthermore, it exerts several biological properties including antimicrobial and antioxidant activity [3]. On the other hand, peppermint is known as a medicinal plant. It is characterized by a refreshing flavor derived from its essential oil of which 30–55 and 14–32% are menthol and menthone, respectively [4]. Menthol is responsible for cooling sensation which arises upon consumption of peppermint [5]. In addition, peppermint possess antifungal, insecticidal, antiviral, antidiabetic, antimicrobial and antioxidant activities [6, 7]. Because of their biological activities and good organoleptic properties, both spearmint and peppermint are used in medicinal and non-medicinal purposes, especially as herbal teas in food industry.

Teas from *Camellia sinensis* and herbal substances are important sources of bioactive compounds in our diet. In addition, ready to drink iced teas of *C. sinensis* have been marketed for decades. However, this option is very rare for other herbal substances. Iced tea falls into ready to drink beverage category which is served to the consumers in either cans or bottles. It can be described as a non-carbonated sugar-sweetened beverage. Iced tea is commonly produced from *C. sinensis* and it contains a certain amount of caffeine which might have adverse effects on human health [8]. It is

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possible to produce caffeine-free iced teas from different herbal substances as alternatives to *C. sinensis* iced teas. For example, ready to drink rooibos iced tea is consumed as a caffeine-free alternative to *C. sinensis* iced tea in South Africa [9]. In the literature, there are numerous studies regarding iced teas obtained from *C. sinensis* [10–14]. However, studies on herbal iced teas are limited to rooibos tea [9, 15–17] and *Spergularia rubra* [18].

Lemon (*Citrus limon*), a rich source of bioactive compounds, contains citric acid, ascorbic acid and flavonoids which can provide numerous health promoting effects [19, 20]. Limonene which provide lemon-like odor is the major compound of lemon essential oil [21]. Lemon juice is commonly added into herbal teas by the consumers. This tradition may enhance the sensory properties and the bioactivities of herbal teas. Several studies have been conducted to improve the biological activities of beverages by adding fruit juices [22]. Lemon flavored iced teas are commercially available in the market [23].

To the best of our knowledge, no previous study was conducted on the production and characterization of iced teas from spearmint and peppermint. Therefore, the objectives of the present study were to (i) produce spearmint and peppermint iced teas using microencapsulated extracts, (ii) determine physicochemical, bioactive and sensory properties of iced teas and (iii) evaluate the effects of the use of microencapsulated lemon extract (MLE) instead of citric acid on properties of the iced teas.

Materials and methods

Materials

Spearmint leaves in dried form and peppermint essential oil were purchased from a herbal store (Arifoğlu, Kayseri, Turkey). Fresh peppermint leaves were supplied from Erciyes University Agricultural Research Center (Kayseri, Turkey) in 2014. Peppermint leaves were dried under shade at ambient temperature for a week and the leaves were separated from the stems after drying. Lemon samples and sugar were purchased from a local market (Beğendik, Kayseri, Turkey). All solvents used were of HPLC grade and other chemicals were of analytical grade. The chemicals, standards, coating materials and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Extraction of phenolic compounds and essential oil from herbs

Pressurized hot water extraction technique was applied using a Dionex ASE 350 (Thermo Scientific, Germany) system with a fixed pressure of 10.34 MPa for the simultaneous extraction of phenolics and essential oils from the herbs. The extraction conditions were reported in detail for spearmint [24] and peppermint [25]. In brief, 10 g spearmint was extracted with pressurized water at 140 °C for 10 min by applying two extraction cycles. The same extraction conditions were applied for peppermint except for the temperature, which was 130 °C. The extract was stored at − 18 °C until further processing.

Extraction of polyphenols and citric acid from lemon

Fresh lemon samples (1 kg) were sliced, and then homogenized with a Waring blender (Staufen, Germany). Homogenized lemon samples were transferred into a 2 L glass beaker filled with 1.5 L of deionized water. The extraction process was conducted with this mixture at 100 °C for 10 min. The insoluble solid particles in the extract were removed by centrifugation (Model NFR800R, Nüve, Ankara, Turkey) for 10 min at 7000×*g*. The resulting supernatant was then concentrated by a rotary evaporator (Heidolph, Schwabach, Hei-Vap Value G1) under vacuum at 40 °C and stored at − 18 °C until further processing.

Preparation of liquid feeds and microencapsulation by spray drying

For liquid feed preparation from spearmint and peppermint extracts, maltodextrin having DE of 13–17 was used as the coating material. The extracts obtained by PHWE (8% solid content) and maltodextrin (8 g) were mixed at a ratio of 1:1 (w/w).

To produce microencapsulated lemon extract (MLE), the concentrated lemon extract (10% solid content), the ratio of lemon extract to gum Arabic (10 g) was adjusted to 1:1 (w/w).

For the production of microencapsulated peppermint essential oil (MPEO), peppermint essential oil and gum Arabic (1:2, w/w) was prepared by dissolving the material at 30 g in 100 mL deionized water.

All the above-mentioned liquid feeds were homogenized under continuous mixing at 1420×*g* for 5 min with an Ultra-Turrax homogenizer (Model T18, Staufen, Germany). Spray dryings were conducted with 200 mL of the liquid feeds using a mini spray dryer (Buchi-B290, Flawil, Switzerland). The drying conditions were as follows: inlet air temperature of 160 °C, aspiration rate of 100%, air flow rate of 600 L/h and feed rate of 8 mL/min.

Iced tea formulations

Iced teas were prepared according to the production principles of commercial iced teas consumed in the Turkish market. Iced teas were labelled as follows: F1

(Microencapsulated spearmint extract + citric acid), F2 (Microencapsulated spearmint extract + MLE), F3 (Microencapsulated peppermint extract + citric acid + MPEO), F4 (Microencapsulated peppermint extract + MLE + MPEO). All formulations contained 7 g sucrose. The formulations of iced teas are summarized in Table 1.

In iced teas enriched with MLE, it was aimed to improve the phenolic content of iced teas and to use natural citric acid from MLE. For this purpose, 1 g MLE, which is equivalent to 0.15% (w/v) citric acid according to results of the informal sensory panel, was added to iced teas (F2 and F4) instead of citric acid. The prepared iced teas were filled into sterile 250 mL glass bottles and pasteurized for 5 min at 80 °C. All iced teas were stored at 4 °C until analyses.

Analyses of color, turbidity, soluble solid content and pH

Color analyses (CIE L*a*b*) as L* (lightness), a* (red to green), and b* (yellow to blue) were carried out using a chromameter (Minolta CR103, Minolta, Japan). Iced tea (20 mL) was transferred to the sampling cell of the equipment. The equipment was calibrated by a white tile before the measurements. Turbidity was measured using a turbidimeter (Hach, Loveland, CO, USA) and the results were expressed in Nephelometric Turbidity Unit (NTU). A refractometer (PAL-3, Atago Instruments, Tokyo, Japan) was used to measure soluble solid content of the iced teas. The pH values were directly measured by a pH meter (Hanna, Italy).

Determination of total phenolic content

The total phenolic contents (TPC) of iced teas were analyzed using Folin–Ciocalteu's phenol reagent [26]. Briefly, 0.4 mL extract or blank, 2 mL diluted Folin–Ciocalteu's phenol reagent (1/10, v/v) and 1.6 mL 7.5% (w/v) sodium carbonate

Table 1 Composition of iced teas

Component	F1	F2	F3	F4
Microencapsulated powder	0.5 (0.25)	0.5 (0.25)	0.25 (0.125)	0.25 (0.125)
MLE	–	1 (0.5)	–	1 (0.5)
MPEO	–	–	0.025 (0.0083)	0.025 (0.0083)

The compositions are expressed as g component per 100 mL iced tea. Values in parentheses indicate (g/100 mL) the extract concentration excluding the coating materials of ingredients of iced teas

MPEO microencapsulated peppermint essential oil, *MLE* microencapsulated lemon extract, *F1* microencapsulated spearmint extract + citric acid, *F2* microencapsulated spearmint extract + MLE, *F3* microencapsulated peppermint extract + citric acid + MPEO, *F4* microencapsulated peppermint extract + MLE + MPEO

solution were mixed at room temperature and stored at dark for 60 min. The absorbance of the mixture was measured using a UV–VIS spectrophotometer (Shimadzu, Japan) at a wavelength of 765 nm. TPC results were calculated using gallic acid calibration equation ($y = 97.762x + 2.405$) and expressed as mg gallic acid equivalents (GAE) per 100 mL iced tea.

DPPH radical scavenging activity

The radical scavenging effects of the iced teas were measured using 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) assay, according to Brand-Williams, Cuvelier, Berset [27] with slight modifications as described by Çam, Hışıl, Durmaz [28]. Briefly, 3.9 mL $DPPH^{\bullet}$ solution and 0.1 mL extract were mixed at room temperature and kept in the dark for 30 min. The control sample was prepared using 0.1 mL methanol. Spectrophotometric measurements were carried out using UV-VIS spectrophotometer at a wavelength of 515 nm. The DPPH results were calculated as EC_{50} value which is defined as the amount of sample necessary to decrease initial DPPH concentration by 50%. EC_{50} was expressed as mL of iced tea to scavenge a gram of DPPH.

ABTS radical scavenging activity

Trolox equivalent antioxidant capacity (TEAC) of iced teas was tested using 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical [29] with some modifications. Briefly, ABTS radical solution (30 mg) containing 2.45 mM potassium peroxodisulfate was diluted by PBS (Phosphate buffer saline, pH 7.6) solution until reaching the absorbance value of 0.700 (±0.02) at 734 nm. Samples were diluted with PBS by 1:50 or 1:100. Extracts at different concentrations (20–80 µL) were pipetted into the cuvette and the volume of the cuvette was adjusted to 2 mL by adding ABTS radical solution. This mixture was incubated in the dark. Then, the absorbance value was measured at 734 nm after 6 min. The results were calculated using Trolox calibration equation ($y = 0.1296x - 0.0394$) and reported as mg Trolox equivalents (TE) per 100 mL iced tea.

UPLC analysis of phenolic compounds

An Ultra performance liquid chromatography (UPLC) method coupled with a diode array detector (DAD) was performed with a chromatograph from Shimadzu (Japan). A mixture of water/acetic acid (98/2, v/v) (A) and acetonitrile (B) were used in a gradient way by keeping the mobile phase flow rate as constant at 0.5 mL/min. The binary gradient followed as: (i) 0–5 min: 95% (A), 5% (B); (ii) 5–25 min: 65% (A), 35% (B); (iii) 25–28 min: 10% (A), 90% (B); (iv) 28–30 min: 95% (A), %5 (B). The column was Acclaim 120 C18

(150 × 2.1 mm, 3 µm, Thermo Scientific) and the column temperature was maintained at 35 °C. The UV–Vis spectra were recorded between 200 and 600 nm. The chromatogram was recorded at 280, 323, 329 and 350 nm, respectively for eriocitrin, caffeic acid, rosmarinic acid and luteolin derivatives. The samples were filtered through a 0.45 µm before analyzed. The limits of detection (LOD) and quantification (LOQ) were calculated from calibration curves. The LOD and LOQ were determined at 3.3 S_a/b and 10 S_a/b, respectively, where S_a is the standard deviation of the residuals and b is the slope of the regression line. Quantification was based on external standard method.

HPLC analysis of citric acid

Analysis of citric acid was performed using an HPLC (Shimadzu, Japan) combined with DAD. The extraction of citric acid from iced teas was carried out according to a method described by Poyrazoğlu, Gökmen, Artık [30]. Separations were performed on a Zorbax C18 column (25 cm × 4.6 mm, 5 µm) using 0.2 M aqueous *meta*-phosphoric acid as the mobile phase at 25 °C with a flow rate of 1.2 mL/min. The chromatogram was monitored at 210 nm. Quantification was based on external standard method.

HPLC analysis of sugars

Sugars were analyzed using an HPLC (Shimadzu, Japan) coupled with a refractive index detector (Shimadzu RID-10A, Japan). A Zorbax carbohydrate analytical column (150 × 4.6 mm, 5 µm) was used to separate the individual sugars (glucose, fructose and sucrose) using acetonitrile-water (75/25, v/v) as the mobile phase at a flow rate of 1 mL/min [31]. The detector was stabilized by passing the mobile phase for 30 min. The iced tea samples were filtered through 0.45 µm before analyzed. Quantification was based on external standard method.

Analyses of volatile compounds

For volatile compound analysis, 2 mL iced tea sample was transferred into a 15 mL headspace vial, and DVB/CAR/PDMS coated fiber (Supelco, Bellafonte, USA) in manual solid phase microextraction (SPME) holder was used to adsorb headspace volatiles of the iced teas. The fiber (preconditioned at 250 °C in injection port of GC for 15 min) was exposed to the headspace at 40 °C for 30 min. The fiber was replaced into the injection port of GC, and the adsorbed components were desorbed at 260 °C for 5 min.

Quantitative and qualitative analysis of volatile components in iced teas were carried out with a gas chromatography–flame ionization detector (GC–FID). GC–FID analyses were carried out on an Agilent 6890N chromatograph fitted with TR5-MS column (60 m x 0.25 mm, 0.25 µm) from Agilent. The oven temperature was kept at 40 °C for 2 min, increased with a gradient of 25 °C/min from 40 to 100 °C. A second gradient was applied to 280 °C at 7 °C/min and then 5 min isotherm at 280 °C. Total analysis time was 50 min. The injector and detector were operated at 260 and 280 °C, respectively. Split ratio was set to 1:10. Hydrogen (Purity 99.999%) was used as carrier gas at a flow rate of 1 mL/min. Quantification was based on external standard method.

Sensory analysis

A trained panel was used for the descriptive sensory profile of iced teas. The panel consisted of four females and two males aged between 24 and 37. The panelists were trained until they understood the descriptive sensory characteristics given in Table 2 and showed consistent replicates. Preparation of reference samples for descriptive terms and the iced teas for sensory analysis were conducted according to relevant literature [32–35]. The sensory panel was developed fifteen sensory descriptors grouped under the following four categories-taste, odor, flavor and overall acceptability. The trained panelists consumed water and crackers between the assessments. Responses were evaluated by a 10-point scale, where 0 for nonexistence and 10 for strong presence of related characteristics.

Statistical analysis

Production of iced tea was performed in duplicate whereas all analyses were performed minimum in triplicate. The data was subjected to analysis of variance (ANOVA), and appropriate mean separation was conducted using Tukey's test or Student t test ($P < 0.05$). All statistical calculations were performed using SPSS 22 statistical package for Windows (SPSS Inc., Chicago, IL, USA).

Results and discussion

Formulations and sensory acceptability of iced teas

In the present study, the main attempt was to produce ready to drink iced teas from microencapsulated spearmint and peppermint extracts. In preliminary study on iced tea formulations, amounts of microencapsulated extracts, sugar and citric acid were evaluated, respectively, in the range of 0.1–1, 6–7.5 and 0.13–0.20 g/100 mL by sensory analysis (data not shown). Sweetness, acidity and flavor perceptions in beverages are linked each other [36]. Thus, ideal amounts of ingredients must be selected in beverage formulations. In the sensory analysis, the amounts of sucrose and citric acid were determined as 7 and 0.15

Table 2 Descriptive terms, definition, and standards used for the training of the panelists

Category	Sensory properties	Descriptions	Standards
Taste	Sweetness	Intensity of taste caused by the sugars	7% sucrose solution
	Bitterness	Bitter flavor from caffeine	0.07% caffeine solution
	Sourness	Sour taste of citric acid	0.2% citric acid solution
Odor	Peppermint odor	Distinctive menthol odor	Peppermint essential oil
	Spearmint odor	Distinctive carvone odor	Spearmint essential oil
	Lemon odor	Lemon-specific odor	1% lemon essential oil
Flavor	Menthol flavor	The flavor associated with menthol	0.04% menthol solution
	Carvone flavor	The flavor associated with carvone	0.05% carvone solution
	Lemon flavor	The flavor associated with lemon	1% lemon essential oil
	Astringency	Astringency flavor from tannins	0.5% punicalagin solution
	Lack of freshness	Absence of minty flavor	Iced tea (Spearmint and peppermint) held for 1 week
	Unnatural flavor	Detection of any flavor in iced teas other than iced tea ingredients.	1% clove in iced tea
	Nasal cooling	Cooling in the end of exposure to the menthol	0.08% menthol solution
	Oral cooling	Cooling effect in the mouth cavity	0.07% menthol solution

g/100 mL in iced teas, respectively. In addition, acceptable amounts of microencapsulated extracts were 0.5 g for spearmint and 0.25 g for peppermint in 100 mL iced tea. The higher or lower amounts of the ingredients in formulations resulted in decrease of acceptance of the iced teas. Sensory acceptability of beverage rich in bioactive compounds is one of the important factors. The more the added amount of herbal extracts into the processed beverage the higher the bioactive properties of the beverage because of phenolic compounds in the herbal extracts. However, an increase in the proportion of phenolic compounds may result in a decrease in the acceptability of the products by the consumers [37]. This phenomenon was valid in the present study as well, therefore, the amounts of herbal extracts in microencapsulated form were kept at 0.25–0.50 g/100 mL levels in the iced teas. Similar findings were reported by de Beer et al. [9] who suggested the use of low concentrations of rooibos extract in the iced teas in order to avoid such problems.

During these preliminary experiments, it was also observed that menthol content of microencapsulated peppermint extracts was below its sensory threshold, which might be due to the loss of menthol during spray drying of the extracts. The loss of menthol could be attributed to maltodextrin used as a coating material. Maltodextrin has some shortcomings in microencapsulation of essential oils because of its low emulsifying properties [38]. Hence, MPEO, produced using gum Arabic as coating material, was added into peppermint iced tea formulations (F3 and F4). However, it was not necessary to add spearmint essential oil to spearmint iced teas.

Physicochemical characteristics

The values of L*, a* and b* in the iced teas ranged from 71.96 to 85.95, − 0.85 to 4.28, and 23.67 to 44.23, respectively (Table 3). Among iced teas, the highest L* values were observed in peppermint iced teas (F3 and F4) which could be explained by lower amounts of microencapsulated extracts in formulations. Because the amount of microencapsulated extracts in spearmint iced teas was twofold the amount of microencapsulated extracts in peppermint iced teas (Table 1). When compared spearmint iced teas, the lowest L* (lightness) values were found in F2, followed by F1. This increase in the value of L* was due to the addition of MLE. Similar findings were also observed in peppermint iced teas. Spearmint iced teas had red (+ a*) color parameters whereas peppermint iced teas showed green (-a*) color parameters. In addition, all iced teas showed + b* values, in accordance with their yellow colors. The use of MLE in formulations increased b* value of iced teas.

The degree of turbidity is also an important parameter reflecting the quality of the beverages. Turbidity values of the iced teas decreased in the descending order: F2 > F1 > F4 > F3. Spearmint iced teas were characterized by their higher turbidity values than peppermint iced teas (Table 3). The turbidity of iced teas significantly ($P < 0.05$) increased with the addition of MLE because MLE did not fully dissolve in iced teas. Nevertheless, soluble solid contents of the iced teas changed between 7.2 to 8.1 g/100 mL, reflecting approximately amounts of ingredients in iced teas.

Hydrolysis of sucrose into invert sugar was observed during the production of iced teas. As a result of this hydrolysis reaction, a mixture of fructose-glucose-sucrose formed in

Table 3 Physicochemical characteristics of iced teas

Analysis	F1	F2	F3	F4
L*	75.03 ± 0.85^{b}	71.96 ± 0.45^{a}	85.95 ± 0.03^{d}	81.07 ± 0.34^{c}
a*	2.81 ± 0.25^{c}	4.28 ± 0.08^{d}	-0.85 ± 0.03^{a}	-0.13 ± 0.10^{b}
b*	39.53 ± 0.30^{c}	44.23 ± 0.09^{d}	23.67 ± 0.06^{a}	31.65 ± 0.14^{b}
Turbidity (NTU)	204.00 ± 4.24^{c}	230.50 ± 0.71^{d}	47.20 ± 0.14^{a}	96.15 ± 0.21^{b}
Citric acid (g/100 mL)	0.14 ± 0.01^{b}	0.19 ± 0.01^{c}	0.13 ± 0.00^{a}	0.18 ± 0.00^{c}
Fructose (g/100 mL)	0.18 ± 0.09^{a}	0.17 ± 0.13^{a}	0.48 ± 0.08^{a}	0.41 ± 0.42^{a}
Glucose (g/100 mL)	0.27 ± 0.10^{a}	0.24 ± 0.09^{a}	0.47 ± 0.15^{a}	0.42 ± 0.19^{a}
Sucrose (g/100 mL)	6.50 ± 0.18^{b}	6.20 ± 0.34^{ab}	5.77 ± 0.32^{a}	5.88 ± 0.46^{ab}
Soluble solid content (g/100 mL)	7.5 ± 0.0^{b}	8.1 ± 0.0^{d}	7.2 ± 0.0^{a}	8.0 ± 0.0^{c}
pH	3.70 ± 0.00^{ab}	3.99 ± 0.00^{b}	3.45 ± 0.00^{a}	3.86 ± 0.00^{ab}

Values that are followed by different letters within each line are significantly different ($P < 0.05$)

MPEO microencapsulated peppermint essential oil, *MLE* microencapsulated lemon extract, *F1* microencapsulated spearmint extract + citric acid, *F2* microencapsulated spearmint extract + MLE, *F3* microencapsulated peppermint extract + citric acid + MPEO, *F4* microencapsulated peppermint extract + MLE + MPEO

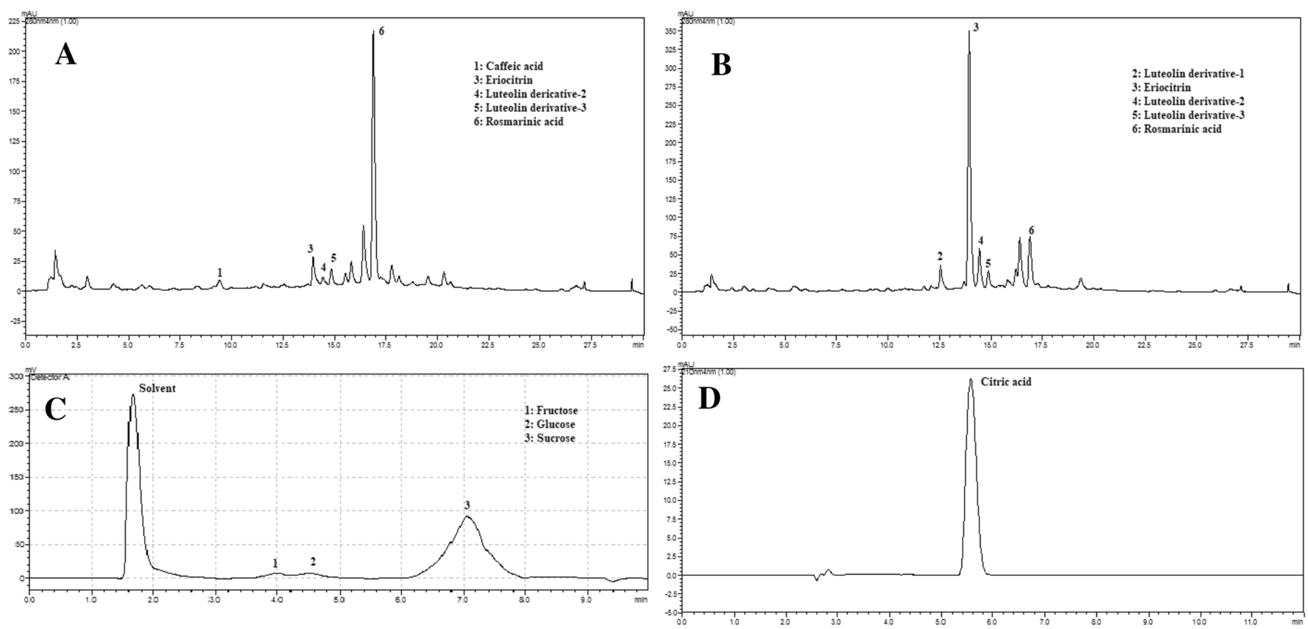

Fig. 1 Representative HPLC chromatograms of phenolics (Spearmint, **a**; Peppermint, **b**), sugars (**c**) and citric acid (**d**) in iced teas

all iced teas (Fig. 1). This finding could be attributed to the acidic nature of the iced teas and pasteurization treatment (5 min at 80 °C) in the production of iced teas. The formation of invert sugar was higher in peppermint iced teas when compared to spearmint iced teas (Table 3). Similar results were observed by de Beer et al. [39] who reported sucrose hydrolysis in functional iced tea beverage powders containing sucrose and citric acid in the presence of moisture. Sucrose is the reference for the sweetness of natural or synthetic sweeteners [40, 41]. Several studies have been conducted to evaluate alternative sweeteners instead of sucrose to obtain healthier products owing to several health problems [42]. However, the replacement of sucrose with natural or artificial sweeteners can cause unpleasant sensory properties such as aftertaste and bitterness [43], and consequently the sensory properties of the products. Thus, sucrose was preferred as sweetener in these novel iced tea formulations.

The citric acid in iced tea was used to inhibit microbial growth and prove acidic taste (sourness). According to the results of the preliminary study, the amount of citric acid was selected as 0.15 g per100 mL iced teas (Table 1). The citric acid content of iced teas with MLE was higher than that of iced teas with citric acid (Table 3). This concentration level of citric acid in iced teas provided pH level below 4.5, thus less thermal load was necessary to assure microbiological safety of the products compared to the counterparts having higher pH values. The highest and lowest pH values were 3.99 in F2 and 3.45 in F3, respectively. In commercial iced teas, pH values were reported as 3.72–4.11 [23] and 2.89–4.03 [44].

Polyphenols in iced teas

The polyphenols in iced teas were analyzed by an UPLC (Fig. 1) and results are shown in Table 4. The results showed that the dominant phenolic compound was rosmarinic acid in spearmint iced teas (F1 and F2), whereas eriocitrin was the main phenolic of peppermint iced teas (F3, F4). A previous study reported that rosmarinic acid and its derivatives were approximately 88% of 66 phenolic compounds identified in aqueous extract of spearmint [45]. In addition, eriocitrin and rosmarinic acid were main phenolics of peppermint in the study of Areias, Valentão, Andrade, Ferreres, Seabra [46]. Our findings are in good agreement with these previous results.

Three peaks in peppermint iced teas and two peaks in spearmint iced teas showed similar UV spectra with luteolin-7-glucoside and they were tentatively qualified as luteolin derivatives. These compounds represented about 30%, 27%, 40% and 41% of identified polyphenols in F1, F2, F3 and F4, respectively. It was previously reported the presence of luteolin derivatives such as luteolin-8-*C*-glucoside, luteolin-rutinoside, luteolin-hexoside, luteolin-7-glucuronide in both spearmint and peppermint [45, 47]. Caffeic acid was also detected in minor amounts in only spearmint iced teas.

Incorporation of MLE into the iced teas resulted in significant increase in the amount of eriocitrin. However, it did not affect the content of other phenolic compounds identified in the present study, except for luteolin derivative-1 in peppermint iced tea. These results are supported by the study of González-Molina et al. [48] demonstrating the presence of eriocitrin in lemon juice. The main phenolic compounds in the iced teas remained the same with the addition of MLE. Catechins were main bioactive compounds in commercial iced teas [49] because green, black or oolong tea extracts were used in the production of many commercial iced teas. In addition, aspalathin in rooibos iced tea was main phenolic compound [9].

Total phenolic contents and antioxidant activities

TPCs of iced teas ranged between 27.37 and 46.31 mg GAE/100 mL samples (Table 5). TPCs of iced teas with

Table 4 Contents of polyphenols in iced teas

Phenolic compounds (mg/L)	Iced teas				Validation parameters			
	F1	F2	F3	F4	Regression equation	R^2	LOD (mg/L)	LOQ (mg/L)
Caffeic acid	3.31 ± 0.87^a	3.78 ± 0.25^a	n.d.	n.d.	y = 0.00019x + 3.56	0.9951	0.01	0.02
Luteolin derivative-1	n.d.	n.d.	17.54 ± 5.90^a	41.10 ± 1.53^b	y = 0.00107x + 3.43	0.9966	0.03	0.10
Eriocitrin	5.26 ± 0.22^a	64.11 ± 4.03^b	124.91 ± 2.44^c	185.01 ± 5.22^d	y = 0.00057x − 20.12	0.9955	0.02	0.05
Luteolin derivative-2	16.19 ± 7.58^a	26.35 ± 2.41^a	62.16 ± 6.23^b	76.21 ± 2.30^b	y = 0.00107x + 3.43	0.9966	0.03	0.10
Luteolin derivative-3	22.21 ± 10.91^a	29.57 ± 8.37^a	26.81 ± 5.27^a	39.73 ± 2.98^a	y = 0.00107x + 3.43	0.9966	0.03	0.10
Rosmarinic acid	82.04 ± 3.79^b	82.59 ± 2.84^b	35.32 ± 2.97^a	38.94 ± 3.97^a	y = 0.00036x + 45.98	0.9982	0.02	0.07

Values that are followed by different letters within each line are significantly different ($P < 0.05$).

n.d. Not detected, *LOD* limit of detection, *LOQ* limit of quantification, *MPEO* microencapsulated peppermint essential oil, *MLE* microencapsulated lemon extract, *F1* microencapsulated spearmint extract + citric acid, *F2* microencapsulated spearmint extract + MLE, *F3* microencapsulated peppermint extract + citric acid + MPEO, *F4* microencapsulated peppermint extract + MLE + MPEO

Table 5 Antioxidant activities and total phenolic contents of iced teas

Antioxidant activity	F1	F2	F3	F4
ABTS (mg TE/100 mL)	61.53 ± 3.08^a	80.88 ± 3.99^b	55.33 ± 0.12^a	77.52 ± 3.32^b
EC_{50} (mL/g DPPH)	402.63 ± 3.49^{ab}	389.12 ± 10.79^a	481.18 ± 7.46^c	470.88 ± 32.07^{bc}
TPC (mg GAE/100 mL)	37.00 ± 0.82^b	46.31 ± 0.63^c	27.37 ± 0.09^a	35.47 ± 0.19^b

Values that are followed by different letters within each line are significantly different ($P < 0.05$)

TE Trolox equivalents, *TPC* total phenolic content, *GAE* gallic acid equivalents, *MPEO* microencapsulated peppermint essential oil, *MLE* microencapsulated lemon extract, *F1* microencapsulated spearmint extract + citric acid, *F2* microencapsulated spearmint extract + MLE, *F3* microencapsulated peppermint extract + citric acid + MPEO, *F4* microencapsulated peppermint extract + MLE + MPEO citric acid (F1 and F3) were lower than that of the iced teas with MLE (F2 and F4) ($P < 0.05$). Large differences were reported for TPCs of iced teas in the literature. For example, when eleven commercial iced teas were analyzed, the iced teas showed variable TPC in the range of 0–99.0 mg/L [50]. TPCs of commercial iced teas in the US market ranged from 40 to 90 mg GAE/100 mL [51]. In addition, TPCs of ready-to-drink flavored and colored commercial teas were reported in the range of 32.35–211.56 mg/L [23]. Similar results were reported for green and fermented rooibos iced teas ranging from 33.81 to 50.88 mg GAE/100 mL [17].

The antioxidant activities of the iced teas were determined by two different methods (Table 5). The ranges of EC_{50} and TEAC values were 389–481 mL/g DPPH and 55.33–80.88 mg/100 mL, respectively. Among the iced teas, F2 displayed the highest antioxidant activity. The use of MLE in formulations improved ABTS radical cation antioxidant capacity and DPPH radical scavenging activity of iced teas. In a previous study, antioxidant activities of blueberry, citrus, lemon, peach, rose petal and sangria flavored teas were tested and lemon-flavored tea showed highest antioxidant activity [23]. This observation was attributed to the antioxidant vitamins in lemon juice by the authors. In addition, TEAC values of commercial iced teas were reported between 27.53 and 262.81 mg/100 mL by Seeram et al. [51]. According to our results and other studies, TPC and antioxidant activity of iced teas depended on herbal materials or other ingredients used in production.

Volatile compounds

Volatile compounds of the iced teas were determined by SPME-GC–FID analysis and chromatograms are presented in Fig. 2. Previous studies reported that carvone and menthol were the major volatile compounds of the essential oils of spearmint and peppermint, respectively [2, 4, 52]. In this stage of our study, it was aimed to measure the amounts of main volatile compounds of spearmint (carvone) and peppermint (menthol, menthone and methyl acetate) corresponding to the amounts of microencapsulated extracts selected in the informal sensory analysis. The amounts of carvone in spearmint iced teas were 0.32 ± 0.03 and 0.30 ± 0.02 mg/100 mL for F1 and F2, respectively. Menthol, menthone and methyl acetate were analyzed for peppermint iced teas. Sum of these

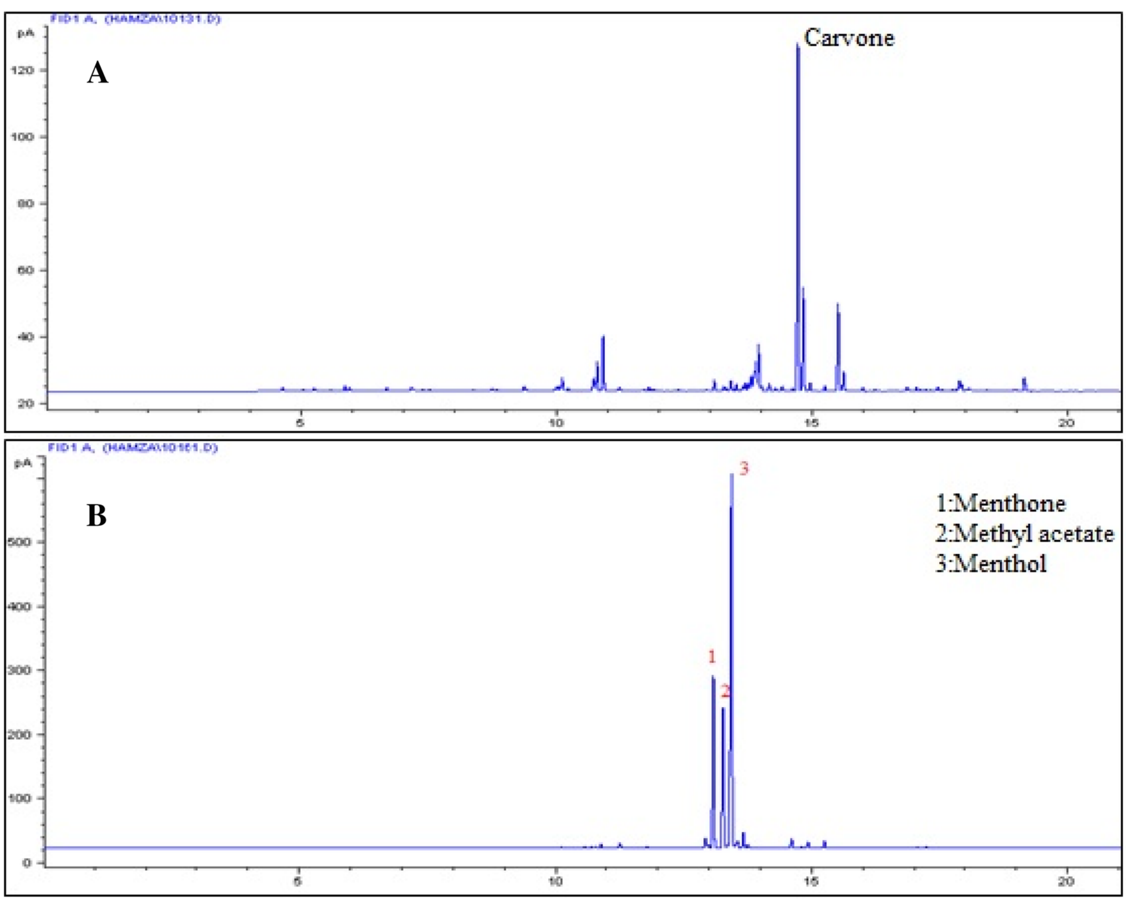

Fig. 2 GC–FID chromatograms of volatile compounds in spearmint (**a**) and peppermint (**b**) iced teas

Table 6 Sensory characteristics of iced teas

Category	Sensory properties	F1	F2	F3	F4
Taste	Sweetness	6.6 ± 1.7^{a}	6.3 ± 1.7^{a}	6.5 ± 1.1^{a}	6.3 ± 1.3^{a}
	Bitterness	n.d.	n.d.	n.d.	n.d.
	Sourness	4.8 ± 1.3^{a}	5.2 ± 1.6^{a}	4.9 ± 1.9^{a}	5.6 ± 1.7^{a}
Odor	Peppermint odor	n.d.	n.d.	7.7 ± 1.1a	7.4 ± 0.5a
	Spearmint odor	6.9 ± 1.0^{a}	6.8 ± 0.8^{a}	n.d.	n.d.
	Lemon odor	5.0 ± 0.7^{a}	5.4 ± 0.9^{a}	4.0 ± 1.0^{a}	5.2 ± 0.4^{a}
Flavor	Menthol flavor	n.d.	n.d.	7.2 ± 1.3^{a}	7.6 ± 0.9^{a}
	Carvone flavor	7.2 ± 0.8^{a}	7.0 ± 1.2^{a}	n.d.	n.d.
	Lemon flavor	4.4 ± 2.6^{a}	5.6 ± 1.1^{a}	4.2 ± 0.8^{a}	5.2 ± 0.8^{a}
	Astringency	n.d.	n.d.	n.d.	n.d.
	Lack of freshness	n.d.	n.d.	n.d.	n.d.
	Unnatural flavor	n.d.	n.d.	n.d.	n.d.
	Nasal cooling	n.d.	n.d.	5.0 ± 2.1^{a}	4.6 ± 2.3^{a}
	Oral cooling	n.d.	n.d.	6.4 ± 1.5^{a}	6.6 ± 0.9^{a}
Overall	Acceptability	8.0 ± 1.0^{a}	7.7 ± 0.7^{a}	7.9 ± 1.1^{a}	7.9 ± 0.5^{a}

Means scores with the same letters are not statistically significant ($P > 0.05$). The sensory analysis was carried out by six trained panelists. Define numbers here 0 and 10 means nonexistence and strong presence of related characteristics, respectively

n.d. Not detected, *MPEO* microencapsulated peppermint essential oil, *MLE* microencapsulated lemon extract, *F1* microencapsulated spearmint extract + citric acid, *F2* microencapsulated spearmint extract + MLE, *F3* microencapsulated peppermint extract + citric acid + MPEO, *F4* microencapsulated peppermint extract + MLE + MPEO

compounds in F3 and F4 was calculated as 6.51 ± 0.74 and 6.75 ± 0.25 mg/100 mL, respectively. There is no reported study regarding the carvone content of spearmint-based beverages. In a study, it was pointed out that peppermint essential oils should be in a balanced manner [53]. Sensorial rejection limits of peppermint essential oil were reported as 1.34 µL per mL of cajá juice and 1.36 µL per mL of guava and mango juices when the essential oils were added into the respective juices. In our study, incorporation levels of essential oils into the iced teas were quite low, and none of the panelists raised an issue of rejection.

Sensory properties

Sensory properties of iced teas are presented in Table 6. The sensory properties of beverages are mainly related to the presence of volatile and non-volatile compounds such as phenolic compounds, organic acid and sugar [10]. In this study, according to panelists' assessment, sensory properties were not significantly affected by MLE addition, even though the average data showed that the iced teas with MLE (F2 and F4) had slightly more sourness, lemon flavor and less sweet than iced teas with citric acid (F1 and F3) (Table 4). The panelists did not find bitterness, astringency, unnatural flavor and lack of freshness for all the iced teas. Lemon odor and flavor were detected not only in the iced teas with MLP but also in the iced teas with citric acid. Lemon odor perception in the iced teas with citric acid could be explained by the presence of limonene in spearmint and peppermint [52]. It was also reported that the effects of sugars and organic acids on flavor perception in a citrus flavored beverage model [54]. Lemon flavor perception might be the presence of citric acid. Nasal and oral cooling sensations were distinctive characteristics of peppermint iced teas. These characteristics were not detected in spearmint iced teas by the panelists.

Conclusions

Microencapsulation is a widely used technique for the protection of bioactive compounds in the food industry and microencapsulated powders are commonly used to enrich food products in terms of functional properties. This study demonstrated the use of microencapsulated extracts as a main ingredient in iced tea production and the suitability of MLE as a substitute for citric acid. The spearmint iced teas had higher TPC than peppermint iced teas. The use of MLE instead of citric acid enhanced TPC and antioxidant properties of iced teas but not affected the sensory attributes. Rosmarinic acid in spearmint iced teas and eriocitrin in peppermint iced teas were main phenolic compounds. MLE addition increased eriocitrin content of the iced teas. Although the iced teas were produced using only sucrose, a mixture of sucrose–fructose–glucose was detected in the iced teas by HPLC analysis. Nasal and oral cooling sensations were identified as descriptive sensory characteristics of peppermint iced teas. Future studies are needed to investigate the changes in physicochemical and bioactive properties that occur in the iced teas before and after pasteurization.

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