PAPER

LEMON VERBENA (*LIPPIA CITRIODORA* KUNTH) BEVERAGES: PHYSICOCHEMICAL PROPERTIES, CONTENTS OF TOTAL PHENOLICS AND MINERALS, AND BIOACCESSIBILITY OF ANTIOXIDANTS

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ABSTRACT

In this study, dried lemon verbena (*Lippia citriodora* Kunth) leaves were used for functional beverage production with addition of sucrose and/or sweeteners. Carbonated or mineral enriched versions of these beverages were also produced. The highest antioxidant bioaccessibility was obtained from sucrose added and natural mineral water enriched beverage both in FRAP (47.01 %) and CUPRAC (11.13 %) assays. In general, all beverages were rich in potassium and the mineral enriched beverages were high in magnesium and calcium. The ascorbic acid value was maximum in carbonated beverages. While energy reduced beverages were rich in total phenolics, sucrose added and natural mineral water enriched beverages showed the highest functionality in terms of bioaccessible antioxidants.

Keywords: antioxidant capacity, bioaccessibility, herbal tea, lemon verbena, total phenolics

1. INTRODUCTION

There has been a growing interest in functional food consumption as a result of an increment in the public awareness on healthy and balanced diet. Functional foods are recognized with their health benefits, which related to the high ratios of bioactive components like ascorbic acid, carotenoids, vitamin E and phenolic compounds (MIRON *et al*., 2013). These natural antioxidants are widely distributed in several parts of higher plants (bark, flowers, fruits, leaf pods, seeds, stems and wood) and have been investigated worldwide. Herbs and spices are among the most important sources of antioxidants and phenolics (YANISHLIEVA *et al*., 2006). Lemon verbena (*Lippia citriodora*), one of these herbal plants, grows spontaneously in South America and is cultivated in North Africa and Southern Europe. It is preferred for refreshing effect, which associated with its lemony flavor since ancient times. Fresh leaves are mainly used as a flavoring agent in fish and poultry dishes, vegetable marinades, salad dressings, jams, puddings, and beverages, while dried leaves are mostly used in herbal teas and sorbets (FUNES *et al*., 2009). Generally, the leaves of this plant are reported to possess digestive, antispasmodic, antipyretic, antioxidant, analgesic, anti-inflammatory, sedative and stomachic properties. In addition, it has been used in infusions for the treatment of asthma, cold, fever, flatulence, colic, diarrhoea and indigestion (RAGONE *et al*., 2007).

Previous studies on lemon verbena mainly concentrated on its chemical characterization and revealed the presence of several phenolic compounds like iridoids, flavonoids, phenolic acids and phenylpropanoids especially verbascoside (FUNES *et al*., 2009). It is well known that the health benefits of polyphenols are proportional with the amount of consumption. The bioaccessibility, the amount of an ingested antioxidative compounds that is available for absorption in the gut after digestion (PALAFOX-CARLOS *et al*., 2011), should be known, since the phytochemicals must be previously available to exert their biological activities (COSTA *et al*., 2014). Bioaccessibility of constituents might be changed according to physical properties and chemical composition of the food, its release from the food matrix, possible interactions with other food components, the presence of suppressors or co-factors and individual digestive capacity (PARADA and AGUILERA, 2007). There is very limited information about the bioaccessibility of antioxidant capacity of herbs or herbal drinks.

Today, herbal tea is traditionally prepared by brewing the fresh or dried leaves, stems, roots or seeds with boiled water or using ready to infuse commercial tea bags. However, brewing methods and parameters of plant species differ from one to another. Due to erroneous brewing practices, the expected health benefits may be minimized and even adverse effects might seen.

The main objective of this study is to produce a new alternative beverage to benefit from the nutritional and functional properties of lemon verbena in different forms. Together with physicochemical properties, total phenolic content of beverages, antioxidant capacity and bioaccessibility of antioxidants were investigated. Furthermore, the optimization of the process parameters with regard to prevent the mistakes applied in traditional techniques and so producing microbiologically safe, standard and value added beverages were aimed in this research.

2. MATERIALS AND METHODS

2.1. Chemicals

All the reagents were analytical grade. TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and bile salts were purchased from Fluka (Switzerland). Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), neocuproine (2,9-dimethyl-1,10-phenanthroline), DPPH (2,2-diphenyl-2-picrylhydrazyl), methanol, sodium carbonate, gallic acid, oxalic acid, nitric acid, sodium bicarbonate and sodium hydroxide were purchased from Sigma Aldrich (Germany). Pepsin, pancreatin, iron (III) chloride hexahydrate, Folin-Ciocalteu reagent, 2,6 dichlorophenol indophenol, copper (II) chloride, ammonium acetate and hydrochloric acid were supplied from Merck (Germany).

2.2. Materials

Lemon verbena was purchased from Kurtsan Food Company (Bursa, Turkey) in the dried form for infusion in the production process of the beverages. Natural lemon flavor was obtained from Aromsa Company (Kocaeli, Turkey) and natural mineral water was acquired from Uludag Beverage Company (Bursa, Turkey).

2.3. Methods

2.3.1 Beverage production

The beverages were produced at a pilot scale. A synthetic cloth bag was used as infuser. The plant was infused (1 %) in a boiled water without additional heating. Then the extract was cooled down to room temperature and used as the main ingredient of the beverages. Afterwards the addition of other ingredients, mixtures were plate filtered (plate filter 60X60 CFP, Zambelli Enotech, Italy).
The brix values of the first group of beverages (sucrose added beverages-SB) were adjusted to 8°±0.5 by using sucrose, citric acid, ascorbic acid and natural lemon flavor. The second group (Energy reduced beverages-EB) was produced using aspartame and acesulfame-K along others. The amount of substituted aspartame and acesulfame–K in place of sugar were calculated according to the relative sweetness values of these sweeteners. Due to the replacement of some sucrose to sweeteners for energy reduction, the brix values of these group beverages were adjusted to 5°±0.5. Four different types of beverage were also produced by carbonation-C (ECB, SCB) and mineral enrichment-M (EMB, SMB). Eventually, six different beverages were formulated (Fig. 1).
The carbonation process was applied to improve the refreshing trait of produced beverages. The antimicrobial agents (Na-benzoate and K-sorbate) were used in these beverages, so they were not subjected to pasteurization process, since CO_2 and antimicrobial agents provided for preservation.
In the mineral enrichment process, the volume of the tap water was reduced by half and the remaining volume was replaced with natural mineral water.
The beverages produced according to these steps mentioned above, were filled into 200 mL glass bottles and pasteurized (except carbonated ones) after capping. The bottles were then cooled and stored at room temperature until analyzed (Fig. 1).

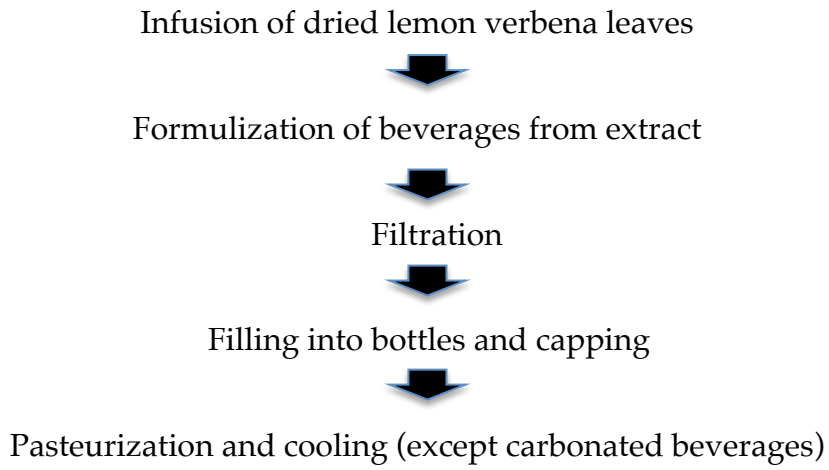

Figure 1. Flow diagram of beverage production.

2.3.2 Evaluation of some physicochemical properties of raw material and beverages

The brix (water soluble dry matter) (AOAC, 1990), titratable acidity and pH (AOAC, 2005), ascorbic acid (SIMONA *et al.*, 2011), color values (*L, a, b*) (BAKKER *et al.*, 1986) and turbidity (TAJCHAKAVİT *et al.*, 2001) analyses were performed on beverages. Moisture content, ascorbic acid, and color (*L, a, b*) were determined in dried lemon verbena leaves to show the physical and chemical properties of raw material. The brix contents of the beverages were analysed using a digital RA-500 model KEM refractometer. Sevencompact pH/Ion Mettler Toledo pH meter, Shimadzu UV 1208 model spectrophotometer, HunterLab Colour Analyzer (MSEZ4500L; HunterLab, Virginia, USA), Hach turbidimeter (Hach, 2100Q) instruments were used for other analyses. All analyses were repeated three times to ensure accuracy of the results.

2.3.3 Mineral content

To determine the quantity of Fe, Ca, Mg and K in raw material and beverages, NMKL (2007) method was applied using Agilent 7500 CX (Agilent Technologies, USA) model ICP-MS. Argon (99.9995 % pure, Linde, Turkey) was used as a carrier gas on ICP-MS and ultra-pure water (18 MΩ·cm at 25 °C resistivity) was generated by purifying distilled water with the New Human Power I (Scholar-UV-PF, 15L/Hr) water purification system. Standard stock solutions containing 1000 mg L^{-1} of each element (Merck, Darmstadt, Germany) were used to prepare the calibration standards. Standards were prepared in 1 % (v/v) HNO_3 on a daily basis.
Approximately 0.5 g of sample was weighed directly in polytetrafluoroethylene (PTFE) flasks after adding 4 mL HNO_3 (Merck Suprapur-65 %) and 1 mL H_2O_2 (Merck Ultrapur-35 %), then flasks were digested in Berghof MWS 3+ (Germany) microwave digestion system. After cooling down to room temperature, the digested sample was transferred to a 50 mL volumetric flask and diluted with distilled water. All samples were filtered with 0.45 μm filters (Hydrophilic PVDF Millipore Millex-HV) prior to the analysis. The instrumental operating conditions for ICP-MS were as follows; Plasma Parameters; RF-Power: 1550 W, Sample depth: 8 mm, Carrier Gas Flow: 0.95 L min^{-1}, Make up Gas Flow: 0.15 L min^{-1},

Nebuliser Pump 0.1 rps, Spray Chamber Temperature: 2 °C, Detector Parameters; Discriminator: 8.0 mV, Analog HV: 1710 V, Pulse HV: 1490 V.
For mineral determination of water used in process, HNO_3 was directly added to water sample. While K ve Ca were analysed by using Eppendorf Elex 6361 model flame photometer, Mg and Fe were analysed with Perkin Elmer Optima 2100 DV model ICP-OES (AYYILDIZ, 1983).

2.3.4 In Vitro Digestion Procedure

To evaluate the functional properties of the beverages, total phenolics, antioxidant capacity and bioaccessibility of antioxidants were investigated. The samples directly taken from each beverage was used for determining the total phenolics and antioxidant capacity. An in vitro digestion enzymatic extraction method, slightly modified version of the one described by VITALI *et al.* (2009) that mimics the conditions in the gastrointestinal tract was used to measure the bioaccessibility of antioxidants. The simulation of gastrointestinal conditions using commercial digestive enzymes (pepsin and pancreatin) is a widely used method for specifying the potential availability of bioactives. Briefly, 10 mL of distilled water and 0.5 mL of pepsin (20 g L^{-1} in 0.1 mol L^{-1} HCl) were added to 1 mL of sample, pH was adjusted to 2 by using 5 mol L^{-1} HCl and sample was incubated at 37 °C in a shaking water bath for 1 h. Simulation of gastric digestion was stopped by the addition of 1 M $NaHCO_3$ (to adjust pH to 7.2). 2.5 mL of bile/pancreatin solution (2 g L^{-1} of pancreatin and 12 g L^{-1} of bile salt in 0.1 M $NaHCO_3$) and 2.5 mL of NaCl/KCl (120 mmol L^{-1} NaCl and 5 mmol L^{-1} KCl) were added to the sample and simulation of intestinal digestion was conducted for the following 2 h. Samples were centrifuged at 3500 rpm for 10 min and the supernatant was used for the analysis. After gastric and intestinal digestion, digested samples were used to determine the bioaccesibility of antioxidants. Bioaccessibility was calculated as the percentage of antioxidant capacity.

2.3.5 Total phenolics

Folin-Ciocalteu reagent was used to determine total phenolics as described by SPANOS and WROLSTAD (1990). In brief, an aliquot (0.25 mL) of sample, 2.3 mL of deionised water and 0.15 mL of Folin-Ciocalteu reagent (FC/Water, 1:5 v/v) were mixed within 10 mL volumetric flask and vortexed for 15 s at room temperature. After 5 min, 0.3 mL of 35 % Na_2CO_3 was added and mixed thoroughly. The absorbance of the mixtures was measured at 725 nm, after incubation for 2 h at room temperature. Water was used as the blank, and gallic acid (GA) solution was used for the calibration of the standard curve (R^2=0.9835). The phenolic content was expressed as gallic acid equivalents (GAE).

2.3.6 Antioxidant capacity

Antioxidant capacity determination methods aim to measure capacity of the antioxidant substances reliably and quickly. So far, various methods were developed, yet only several of them are recommended to be used together to determine the in vitro available antioxidant capacity.

2.3.6.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Antioxidant capacity of the beverages and digested extracts by using DPPH free radical was measured using a modified version of the KATALINIC *et al.* (2006). In this method, the antioxidants were allowed to react with the stable radical in methanolic solution. The

discoloration of the DPPH radicals was monitored through the decrease in absorbance at a characteristic wavelength during the reaction. First, 0.1 mL sample was added to 3.9 mL of 6 x 10^{-5} M methanolic solution of DPPH radical and vortexed (Vortex Mixer Classic, Velp Scientifica, Italia) for 15-30 s. The reaction was allowed to occur in dark at room temperature for 30 mins. A trolox calibration curve (R^2=0.9951) was obtained by measuring the reduction in absorbance of the DPPH solution in 517 nm in the presence of different concentrations of trolox (10-100 μmol L^{-1}).

2.3.6.2 FRAP (ferric reducing antioxidant power) assay

According to BENZIE and STRAIN (1996), 3 mL of daily prepared FRAP reagent (incubated at 37 °C) was mixed with 300 μL of distilled water and 100 μL of the test sample (or extraction solvent for the reagent blank). The test samples, digested extracts and blank were incubated at 37 °C for 60 min. At the end of incubation, absorbance was measured immediately at 595 nm. The FRAP reagent was prepared by mixing 25 mL of 0.3 mol L^{-1} acetate buffer (pH 3.6), 2.5 mL of 20 mmol L^{-1} $FeCl_3$ x 6 H_2O and 2.5 mL 10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1} HCl. The results were calculated from calibration curve as μmol trolox mL^{-1} for beverages (R^2=0.9975).

2.3.6.3 CUPRAC (cupric ion reducing antioxidant capacity) assay

Estimation of cupric ion reducing antioxidant capacity was achieved based on the method of APAK *et al.*, (2008). 1 mL 1 x 10^{-2} M copper (II) chloride + 1 mL 7.5 x 10^{-3} M neocuproine + 1 mL 1 M ammonium acetate were added to x mL 10^{-3} M antioxidant neutral solution + (1-x) H_2O:VT =4 mL; and the final absorbance was measured at 450 nm after 30 min (R^2=0.9947). Calculation of antioxidant capacity was done as trolox equivalents (TEAC values).

2.3.7 Sensory analysis

The beverages were evaluated based on color, odor, appearance and taste by a panel consisting of 10 trained members using a ranking test (ALTUĞ and ELMACI, 2011). According to this test, the panelists ranked the samples from their best favourite one to their least favorite by giving points between 1 and 6. As a result of this statistical test, samples within the range of 22 - 48 did not show any statistical difference while the samples that ranked below 22 (the mean of all of the panelist's point values) were preferred and samples ranked above 48 were rejected at the 95 % probability level ($p < 0.05$).

2.3.8 Statistical analysis

The experiment was conducted in a completely randomized design with three replications. The results were statistically evaluated by one-way analysis of variance (ANOVA) using the JMP software package version 6.0 (SAS Institute Inc. NC, 27513). When significant differences were found ($p < 0.05$), the Least Significant Difference (LSD) test was used to determine the differences among means.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical properties

In this study, the physico-chemical properties of dried lemon verbena leaves and beverages were performed in addition to the bioactive content. The moisture content of dried lemon verbena leaves was measured as 7.24±0.04 g 100 g^{-1}. This result was regarded appropriate by the limits Turkish Standards Institute, which was defined as 10 g 100 g^{-1} max for dried herbs (ANONYMOUS, 2014). EBADI *et al.*, (2015) similarly determined 9 g 100 g^{-1} moisture content in *Lippia citriodora* Kunth leaves with different drying methods.
Physico-chemical properties of lemon verbena beverages are shown in Table 1. All data in Tables are expressed as means±standard deviations (n = 3).
The brix and titratable acidity values of the beverages were adjusted based on the results of market survey on similar beverages conducted prior to the production (ice tea, natural and flavored mineral water, lemonade). The differences between the water soluble dry matter contents of all beverages were found statistically significant ($p < 0.05$) (Table 1). Titratable acidity of the beverages was lower than the sum of citric acid and ascorbic acid added in the production. This decrease could be explained by neutralization of the acidity by the hardness of the water used or the buffer salts of the extract (CEMEROĞLU, 2007). The highest acidity value of ECB could be the result of dissolution of carbondioxide in aqueous medium as carbonic acid (ADEBAYO *et al.*, 2015). The pH values of all beverages were similar with the pH values of some commercial herbal tea beverages determined by PHELAN and REES (2003).

Table 1. Physico-chemical properties of lemon verbena beverages.

Analyses	SB	SCB	SMB	EB	ECB	EMB
Water soluble dry matter (g 100 g^{-1})	8.10±0.00^{a}	7.50±0.01^{c}	7.80±0.00^{b}	5.20±0.00^{e}	5.50±0.10^{d}	4.90±0.00^{f}
Titratable acidity (g 100 mL^{-1})*	0.18±0.00^{b}	0.17±0.01^{c}	0.15±0.00^{d}	0.18±0.00^{b}	0.19.±0.01^{a}	0.14±0.00^{e}
pH	3.32±0.01^{d}	3.62±0.00^{b}	3.79±0.01^{a}	3.33±0.00^{d}	3.54±0.00^{c}	3.79±0.02^{a}
Ascorbic acid (mg 100 mL^{-1})	20.48±0.27^{bc}	28.15±0..30^{a}	20.30±1.60^{bc}	19.36±2.06^{c}	29.57±0.47^{a}	22.19±0.36^{b}
Color						
*L***	11.63±0.15^{cd}	12.20±0.26^{ab}	12.53±0.31^{ab}	12.03±0.49^{bc}	11.47±0.31^{d}	12.70±0.17^{a}
*a***	-1.63±1.00^{a}	-3.73±0.49^{d}	-3.07±0.38^{bcd}	-2.37±0.46^{ab}	-2.60±0.20^{abc}	-3.37±0.49^{cd}
*b***	5.33±0.21b^{c}	6.07±0.11^{a}	4.90±0.40^{c}	5.70±0.20^{ab}	5.70±0.26^{ab}	5.40±0.17^{b}
NTU***	5.14±0.22^{b}	2.40±0.04^{f}	4.02±0.20^{c}	6.00±0.10^{a}	2.86±0.14^{e}	3.38±0.19^{d}

SB: sucrose added beverage; SCB: sucrose added and carbonated beverage; SMB: sucrose added and mineral enriched beverage.
EB: Energy reduced beverage; ECB: Energy reduced and carbonated beverage; EMB: Energy reduced and mineral enriched beverage.
*: Citric acid.
** *L* means lightness of the beverages, and ranges from black to white (0-100). A negative value of *a* indicates green, while *a* positive value indicates red-purple color. Positive *b* indicates yellow and negative blue color.
*** Nephelometric Turbidity Unit.
(Mean values within a column with unlike superscript letters were significantly different ($p < 0.05$))

The ascorbic acid content of beverages was contributed as both antioxidant source and acidity regulator with citric acid. The highest ascorbic acid contents of SCB and ECB samples were related with the production process in which samples were carbonated after addition of antimicrobial agents instead of pasteurization. Therefore, the thermal degredation of ascorbic acid was not occured in these beverages. Reduction of ascorbic acid in other samples was related with the heat treatment (LEŠKOVÁ *et al.*, 2006). The differences between all samples were found to be statistically significant ($p < 0.05$). The ascorbic acid content of lemon verbena leaves were 5.73±0.27 mg 100 g^{-1} and the higher ascorbic acid values of beverages compared to the raw-material in this study can be explained by the intentional addition of ascorbic acid during production. COSTA *et al.*, (2012) reported the ascorbic acid content as 7.20±0.20 mg 100 mL^{-1} in a beverage prepared with the 0.75 % infusion of rooibos red tea leaves (*Aspalathus linearis*) and 21.40±0.10 mg 100 mL^{-1} in a beverage produced with 1 % green tea (*Camellia sinensis*) infusion. SUNA *et al.*, (2016) determined the ascorbic acid content of a mineral enriched *Erica arborea* herbal tea beverage as 28.15±0.30 mg 100 mL^{-1}. The difference between the results might be due to the variety of herb used in the process or its concentration. Additionally, TAMER *et al.*, (2016) determined ascorbic acid content of linden enriched lemonade as 597.9 mg kg^{-1} which was found higher than our results as a consequence of high amount of ascorbic acid coming from lemonade.

According to the results of the statistical analysis, the overall color parameters for beverages are affected significantly by the different production processes ($p < 0.05$). As shown in Table 1, beverages were in green and yellow colour tones. While EMB had the highest *L* (brightness) value, ECB had the lowest value. The highest turbidity value (6.00±0.1) was measured to be the sample EB, which also had the highest total phenolic content (Table 3). It is stated that, chemical turbidity consisted of complexing of some organic compounds like starch, polyphenols, proteins, pectin and minerals like Cu and Fe (SIEBERT, 1999). Accordingly, polyphenols may increase the turbidity by complexing with metals and proteins over time (BEVERIDGE, 1997). The color and turbidity values of the products could not be compared due to the absence of similar beverage.

3.2. Mineral contents

Mineral content of raw-material and beverages are given in Table 2. It is clear that plants take minerals, which are essential for their life-cycle, from soil. The mineral composition of the plants is also affected from the physical and chemical characteristics of soil, usage of natural or artifical fertilizers, storage conditions, climate, region etc. Additionally, the mineral content of the infusions obtained from these plants vary with the mineral amount in leaves and extraction yield (COSTA *et al.*, 2002). The beverages were rich in K, Ca and Mg as the raw material used in their production. The same minerals were found in similar values in herbal tea of *Lippia multiflora* in various researches (TETTEY-LARBI, *et al.* 2015; CHRISTINE, *et al.* 2017). Fe, Ca, Mg and K content of water used in our process was 0.03 mg L^{-1}, 13.0 mg L^{-1}, 1.72 mg L^{-1} and 0.51 mg L^{-1}, respectively. The highest amount of Ca and Mg were determined in SMB and EMB whereas ECB had the highest amounts of Fe and K. While dried lemon verbena leaves and water used in process were rich in Ca, K was higher in beverages. It could be explained with different extraction ratios of minerals. Regarding this issue, PYTLAKOWSKA *et al.* (2012) studied efficiency of mineral extraction from tea leaves and classified the elements in herb infusions as highly-extractable (>55 %) as K; moderately-extractable (20-55 %) including Mg, Na, P, B, Zn and Cu and poorly-extractable (<20 %) comprising Al, Fe, Mn, Ba, Ca and Sr. The researchers also determined the content of some minerals in *Melissa officinalis* infusion (1 %, 10 min brewed) and reported values of 3.90±0.07 $\mu g\ g^{-1}$ (mg kg^{-1}) for Fe, 21.0±0.20 mg kg^{-1} for Ca, 198.00±5.00 mg

kg^{-1} for Mg, 1449.00±12.00 mg kg^{-1} for K, respectively. Similarly, ÖZCAN and AKBULUT (2008) analysed the mineral content of *Melissa officinalis* infusion (2 %) as 20.11 mg 100mL^{-1} for Ca. While our Fe and Ca values were higher, Mg and K contents were lower than these studies. It could be related to raw material and differences in processing conditions.

Table 2. Mineral content of dried lemon verbena leaves (mg kg^{-1}) and beverage samples (mg L^{-1}).

	Fe	Ca	Mg	K
SB	0.11±0.00c	89.72±2.93d	28.87±0.56d	184.35±4.58c
SCB	0.26±0.00b	103.58±1.16c	30.26±0.37c	193.48±2.81b
SMB	0.01±0.00d	122.48±5.17b	43.47±1.29b	121.38±3.37e
EB	0.12±0.05c	88.98±0.74d	28.57±0.25d	197.38±1.10b
ECB	0.37±0.00a	87.51±0.7d	27.98±0.22d	222.28±1.71a
EMB	0.01±0.00d	129.85±1.85a	46.21±0.75a	130.78±1.160d
Raw-material	82.61±1.30	24800±0.02	2814.78±20.00	15600±0.00

*Mean values within a column with unlike superscript letters were significantly different ($p < 0.05$).

3.3. Phenolic contents

Phenolic compounds play an important role regarding in antioxidant effects and defensive action in plants or the human body (BOO *et al.,* 2012). Total phenolic contents of the beverages were given in Table 3.

Table 3. Total phenolic contents of the beverages.

Sample	Total phenolics (mg GAE 100 mL^{-1})
SB	209.35±3.77d
SCB	239.33±14.64c
SMB	232.25±7.67c
EB	360.78±14.11a
ECB	302.55±11.84b
EMB	306.80±7.50b

Mean values within a column with unlike superscript letters were significantly different ($p < 0.05$).

In the average of 2.74-4.71 % of dried lemon verbena polyphenols (7653.46±36.62 mg GAE/100 g^{-1}) were transferred to beverages after extraction process. Energy reduced beverages had generally higher total phenolic contents than sucrose added beverages. Among these EB showed the highest total (360.78±14.11 mg GAE 100 mL^{-1}) phenolic value. Due to the lack of literature data dealing with phenolic content of lemon verbena beverages, our results were compared with polyphenol content of similar types of samples. For instance, DIAS *et al.* (2012) analysed total phenolic content of *Melissa officinalis* infusion (0.50 %) in lyophilized extracts of commercial bag and granulated forms as 959.54±10.02 mg GAE mL^{-1} and 657.06±0.80 mg GAE mL^{-1} respectively, whereas COSTA *et al.*, (2012) determined green tea infusions' (1 %) total phenolic content as 29.10±0.50 mg GAE 100 mL^{-1}. GUIMARÃES *et al.,* (2011) studied infusion and decoction of lemon verbena

and fennel mixed herbs and reported phenolics value of decoction and infusion respectively as 389.73±4.00 mg GAE g^{-1} and 438.08±0.19 mg GAE g^{-1}. ATOUI *et al.*, (2005) also determined total phenolic content of 1.25 % infusion of chinese green tea (*Camellia sinensis*) and greek mountain tea (*Sideritis syriaca*) approximately as 507 mg GA 100mL^{-1} and 37 mg GA 100 mL^{-1}, respectively. Our results were different from the literature data owing to the differences in extraction method, concentration and the material. According to VELIOGLU *et al.*, (1998) antioxidant activity and total phenolics were found to be positively and significantly correlated.

3.4. Antioxidant capacity and bioaccessibility

The antioxidant capacity and the bioaccessibilities of the antioxidant capacity of the beverages are given in Table 4. The differences between the antioxidant capacities of the beverages were significant ($p < 0.05$). The different values obtained from the three assays are due to the different reaction mechanisms or kinetics of the test materials (i.e. DPPH, Cu^{2+} and Fe^{3+}) quenched/reduced by beverages react according to different mechanism and kinetics (JESZKA-SKOWRON *et al.*, 2015). However, the extraction method or differences in concentration make difficult to compare the results. YOO *et al.*, (2008) reported DPPH antioxidant capacity of lemon verbena infusion as 86.90±2.20 %. SB, EB and ECB samples analysed with respectively FRAP, CUPRAC and DPPH assays showed higher antioxidant capacity values than those of others (Table 4).

Table 4. Bioaccessibility of antioxidant capacity of the beverages (µmol trolox mL^{-1}).

Sample	DPPH (µmol trolox mL^{-1*})	DPPH Bioaccessibility (%)	FRAP (µmol trolox mL^{-1*})	FRAP Bioaccessibility (%)	CUPRAC (µmol trolox mL^{-1*})	CUPRAC Bioaccessibility (%)
SB	27.17±0.09a	0.99	32.00±0.78a	19.63	73.35±1.27b	9.26
SCB	23.75±0.70b	0.93	19.97±0.25cd	27.84	62.65±6.89c	10.39
SMB	24.85±2.64b	0.85	17.55±2.35d	47.01	30.46±6.53e	11.13
EB	27.64±0.08a	0.58	26.60±1.82b	32.07	81.72±2.11a	7.13
ECB	27.78±0.30a	0.50	22.61±1.51c	43.74	69.24±0.84bc	7.47
EMB	27.03±0.27a	0.89	19.19±1.72d	37.99	40.83±1.97d	9.65

Mean values within a column with unlike superscript letters were significantly different ($p < 0.05$).

The bioaccesibility ratios were higher in order of FRAP, CUPRAC and DPPH assays. Especially SMB had the highest bioaccessibility obtained from FRAP (47.01 %) and CUPRAC (11.13 %) assays. A possible reason of varying bioaccessibility of antioxidant capacity values could be associated with several factors related to the process conditions, chemical interactions with other phytochemicals, biomolecules present in the food and also the protocols used for the measurements (PARADA & AGUILERA, 2007). The bioaccessibility of antioxidant capacity was decrased in this study. In agreement with our data, HENNING *et al.*, (2014) reported a 21.5 % and 8.1 % decrease of TEAC (trolox equivalent antioxidant capacity) in green tea and grape seed samples during in vitro simulated digestion. In another study, ŞAHAN *et al.*, (2017) reported the bioaccessibility of chicory cultivars with $TEAC_{CUPRAC}$ and $TEAC_{DPPH}$ assays between 62.12–73.48 % and 64.66-76.21 %, respectively. DEĞIRMENCIOĞLU *et al.*, (2016) concluded $TEAC_{CUPRAC}$ bioaccessibility of fermented vegetable juices between 16-36 %. Although the polyphenols supply major antioxidant potency of the samples, our results displayed that digestion may

alter antioxidant properties depending on the variations in polyphenol content (HENNING *et al.,* 2014). Besides, it is known that, structural changes after GI digestion affect both further polyphenol uptake and result in a significant loss of the antioxidant activity (RODRÍGUEZ-ROQUE *et al.,* 2013). Data of this study confirmed that different applications and methods may have an influence on the release of total phenols and their antioxidant capacity, therefore, on the bioaccessible fraction. Likewise, phenolic compounds which demonstrate antioxidant capacity would be bioavailable after digestion and might contribute to protection of humans from several diseases (PÉREZ-VICENTE *et al.,* 2002).

3.5. Sensory findings

Sensory properties of the beverages are shown in Fig. 2. For sensorial test, panelists were briefed about the properties of the beverages. The product should have light yellow color related with the extract and should not contain any particles, while the typical odor and taste of the extract should be appreciated. There were no significant differences in color, odor, appearance and taste values between samples and all of the beverages were generally accepted by the panelists ($p < 0.05$).

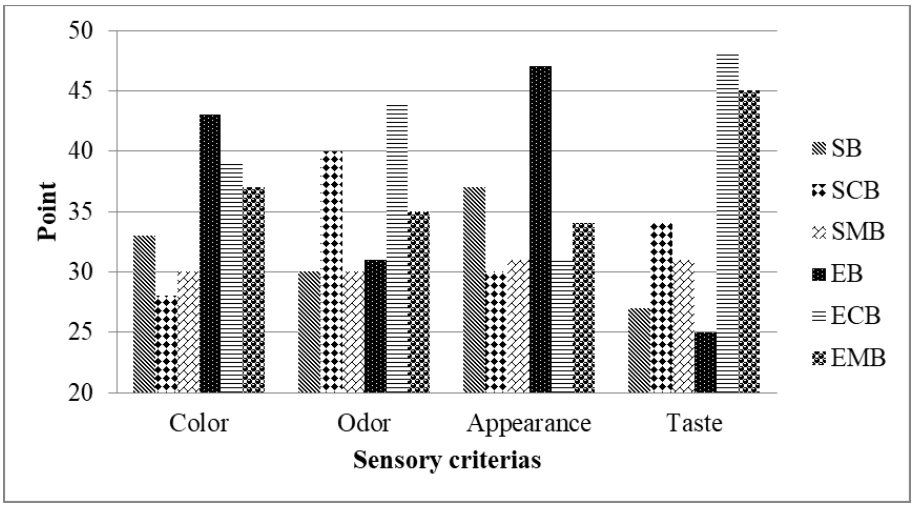

Figure 2. Sensory properties of lemon verbena beverages.

4. CONCLUSIONS

Overall, the sucrose added and also the sucrose added and mineral enriched beverages were found to be the most nutritive beverages among our products because of the highest bioaccessibility values of antioxidant capacity. In general, energy reduced beverages had higher phenolic content. All samples were preferred as sensorial. As a result of growing market interest in functional drinks, natural herbal extracts became popular due to their high bioactive components. In addition, they are easy to formulate and process. Nevertheless, bioaccessibility of a novel herbal tea beverage has not yet been reported in the literature data so far. From this perspective, the bioactive components and their bioaccessibilities in herbal infusions and herbal beverages are needed to be investigated in further studies.

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