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Determination of Physicochemical Properties, Bioaccessibility of Phenolics and Antioxidant Capacity of Mineral Enriched Linden Herbal Tea Beverage

Senem Suna, Canan Ece Tamer, Ömer Utku Çopur

***Abstract*—In this research, dried linden (*Tilia argentea*) leaves and blossoms were used as a raw material for mineral enriched herbal tea beverage production. For this aim, %1 dried linden was infused with boiling water (100 °C) for 5 minutes. After cooling, sucrose, citric acid, ascorbic acid, natural lemon flavor and natural mineral water were added. Beverage samples were plate filtered, filled into 200-mL glass bottles, capped then pasteurized at 98 °C for 15 minutes. Water soluble dry matter, titratable acidity, ascorbic acid, pH, minerals (Fe, Ca, Mg, K, Na), color (L*, a*, b*), turbidity, bioaccessible phenolics and antioxidant capacity were analyzed. Water soluble dry matter, titratable acidity, and ascorbic were determined as 7.66±0.28 g/100 g, 0.13±0.00 g/100 mL, and 19.42±0.62 mg/100 mL, respectively. pH was measured as 3.69. Fe, Ca, Mg, K and Na contents of the beverage were determined as 0.12±0.00, 115.48±0.05, 34.72±0.14, 48.67±0.43 and 85.72±1.01 mg/L, respectively. Color was measured as 13.63±0.05, -4.33±0.05, and 3.06±0.05 for *L*, a*,* and *b** values. Turbidity was determined as 0.69±0.07 NTU. Bioaccessible phenolics were determined as 312.82±5.91 mg GAE/100 mL. Antioxidant capacities of chemical (MetOH:H_2O:HCl) and physiological extracts (in vitro digestive enzymatic extraction) with DPPH (27.59±0.53 and 0.17±0.02 µmol trolox/mL), FRAP (21.01±0.97 and 13.27±0.19 µmol trolox/mL) and CUPRAC (44.71±9.42 and 2.80±0.64 µmol trolox/mL) methods were also evaluated. As a result, enrichment with natural mineral water was proposed for the development of functional and nutritional values together with a good potential for commercialization.

***Keywords*—Antioxidant capacity, bioaccessibility, herbal tea beverage, linden.

I. INTRODUCTION

MEDICINAL and aromatic plants are getting popular day by day between health related consumers. This popularity comes from their biologically active components such as alkoloids, glycosides, essential oils and other bioactive substances. In different countries, medicinal and aromatic plants consumed as a food, nutritional supplement or a raw material for drugs, perfumery and cosmetic industry. *Tilia* genus (*Tiliaceae*) includes nearly 25 species, of which 17 species are present in East Asia, six in Europe, and one in North America [1], [2]. They have often described as species, varieties or forms, and many of them are extensively planted in parks and gardens [3]. In markets, linden flowers are sold with or without bracts and sometimes with leaves under the Turkish name "Ihlamur". The Commission E approved linden flower for colds and cold-related coughs [4]. The British Herbal Compendium indicates its use for upper respiratory catarrh, common colds, irritable coughs, hypertension and restlessness [5]. Vegetables, legumes and whole grain cereals are good sources of antioxidants. Herbal infusions are also important sources [6]. Tea and herbal infusions contribute to the major source of phenolic compounds in our diet [7]. The widespread uses of herbs can be defined such that the people either buy dried parts of herbs from market or they can prefer ready to infuse tea bags containing herbs. Infusion methods and parameters of plants differ from each other, and inappropriate applications minimize expected benefits and may lead to significant adverse health effects.

This study was designed for: preventing mistakes in traditional methods, standardizing herbal tea beverage production, enabling them to be consumed in all seasons, and obtaining a safe product that is reliable on microbial aspects.

II. MATERIAL AND METHODS

Tilia argentea plant was purchased from Kurtsan Food Company in Bursa, Turkey. Dried leaves and blossoms were used as raw material for infusion in the production process of the beverages. Natural lemon flavor was obtained from Aromsa Company in Kocaeli, Turkey and natural mineral water was acquired from Uludag Beverage Company in Bursa, Turkey.

All reagents used were in 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 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).

Senem Suna is with the University of Uludag,, Faculty of Agriculture, Department of Food Engineering, 16059 Gorukle-Bursa, Turkey (corresponding author, phone: +90224 2941492; fax: +90 224 2941402; e-mail: syonak@uludag.edu.tr).

Canan Ece Tamer and Ömer Utku Çopur are with the University of Uludag, Faculty of Agriculture, Department of Food Engineering, 16059 Gorukle-Bursa, Turkey (e-mail: etamer@uludag.edu.tr, ucopur@uludag.edu.tr).

A. Production of Herbal Tea Beverage

Herbal tea beverage was produced in a pilot scale. 500 mL water was boiled and then 10 g of plant material placed in a synthetic cloth bag for infusion. This bag was left to stay in water for 5 min without additional heating. The extract was cooled down to room temperature and used as the main ingredient of the beverage. After the addition of natural mineral water as same quantity of water into infusion, the brix value of the beverage was adjusted to 8°±0.5 with sucrose, citric acid, ascorbic acid and flavor. Then, the mixture was plate filtered, filled into 200 mL glass bottles and pasteurized (98 °C, 15 minutes) after capping. The bottles were then cooled and stored until analyzed. The formulation was determined according to sensorial preferences of the panelists.

B. Physicochemical Analysis

Moisture content, ascorbic acid, color (L^*, a^*, b^*) were analyzed in dried linden leaves and blossoms to express the physical and chemical properties of the raw material. Additionally, water soluble dry matter, titratable acidity, pH, ascorbic acid, color values (L^*, a^*, b^*) and turbidity analysis were conducted in beverage.

Moisture of dried linden leaves and blossoms were determined by oven drying method. Water soluble dry matter of the beverage was expressed as g/100 g by using a digital refractometer (RA-500 model KEM) [8]. Titratable acidity was determined by titrating the sample to pH 8.1 with 0.1 N NaOH and expressed as citric acid content [9]. The pH of the beverage was measured by using a Sevencompact pH/Ion Mettler Toledo pH meter. The ascorbic acid was determined by Shimadzu UV 1208 model spectrophotometer using a 2,6-dichlorophenol indophenol dye and expressed as mg/100 g [10]. Color values were measured using HunterLab Colour Analyzer (MSEZ4500L; HunterLab, Virginia, USA), and the CIE L^*, a^* and b^* values were determined. After initial calibration against standard white and black surface plates, measurements of the sample were performed [11]. Turbidity of the sample was measured as Nephelometric Turbidity Unit (NTU) using a Hach turbidimeter (Hach, 2100Q) [12].

C. Determination of Minerals

For the determination of Fe, Ca, Mg, K and Na minerals in dried leaves, blossoms and beverage, Reference [13] method was employed, and Agilent 7500 CX (Agilent Technologies, USA) model ICP-MS was used. Triplicate measurements were performed for each analysis. Results were expressed as mg/kg in dried linden and mg/L in beverage.

D. Determination of Phenolics and Antioxidant Capacity

In order to assess the functional properties of the beverage, extractable and bioaccessible phenolics were investigated. Extractable phenolics were determined according to Reference [14] with some modifications. For the determination of bioaccessible phenolics, an in vitro digestion enzymatic extraction method was used, that mimics the conditions in the gastrointestinal tract (physiological extract) [14].

The phenolic content was expressed as gallic acid equivalents, mg GAE 100/ mL for the beverage and mg GAE/100 g DW (dry weight) for dried linden (leaves and blossoms).

Antioxidant capacity of extractable and bioaccesible phenolics was determined both in dried linden and beverage using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [15], ferric reducing antioxidant power assay (FRAP) [16] and cupric ion reducing antioxidant activity assay (CUPRAC) [17]. Results were expressed as μmol trolox/ml for the beverage and μmol trolox/g dw (dry weight) for dried linden.

E. Statistical Analysis

Findings of the research were analysed with one-way analysis of variance (ANOVA) (JMP software package version 6.0, SAS Institute Inc. NC, 27513).

III. Results and Discussion

The moisture content of dried linden was measured as 9.73±0.04 g/100 g. This result was appropriate according to Turkish Standards Institution limits which were subjected as maximum 10 g/100g for dried herbs [18]. Ascorbic acid content of dried linden was determined as 2.66±0.09 mg/100 g, and color was measured as 47.77± 0.31, -1.77±0.52, and 16.53±0.19 for L^*, a^* and b^* values. Physico-chemical properties of mineral enriched linden herbal tea beverage (MLTB) were shown in Table I.

TABLE I
PHYSICO-CHEMICAL PROPERTIES OF MINERAL ENRICHED LINDEN HERBAL TEA BEVERAGE (MLTB)

Analyses	Mineral enriched linden herbal tea beverage (MLTB)
Water soluble dry matter (g/100 g)	7.66±0.28
Titratable acidity (g/100 mL)*	0.13±0.00
pH	3.69±0.01
Ascorbic acid (mg/100 mL)	19.42±0.62
Color	
L^*	13.63±0.05
a^*	-4.33±0.05
b^*	3.06±0.05
NTU	0.69±0.07

* Citric acid

Reference [19] determined similar pH values of some commercial herbal tea beverages. Reference [20] investigated ascorbic acid content in several herbal infusions in agreement with our data. L^*, a^* and b^* values of herbal tea beverage were seen in Table I. Turbidity value of sample was 0.69 NTU. Reference [21] determined turbidity value of filtered black tea which ranged between 0.12-2.85 for 30 days of storage. Mineral contents of dried linden leaves and blossoms and herbal tea beverage are given in Table II.

It is stated that, plants take minerals which are essential for their life from soil, and mineral composition of the plants are affected from physical and chemical characterization of soil, usage of natural or artificial fertilizers, storage conditions, climate, region, etc. Additionally, mineral contents of the infusions obtained from these plants are related with the mineral amount in leaves and extraction yield [22]. Reference

[23] studied efficiency of mineral extraction from tea leaves and classified the elements in herb infusions as highly-extractable (>55%) like K; moderately-extractable (20-55%) including Mg, Na, P, B, Zn and Cu and poorly-extractable (<20%) including Al, Fe, Mn, Ba, Ca and Sr.

TABLE II
MINERAL CONTENT OF DRIED LINDEN LEAVES AND BLOSSOMS AND MINERAL ENRICHED LINDEN HERBAL TEA BEVERAGE (MLTB

	Fe	Ca	Mg	K	Na
Dried leaves (mg/kg)	50.05±0.62	15300±0.09	2483.96±55.49	14100±0.06	191.53±4.79
MLTB (mg/L)	0.12±0.00	115.48±0.05	34.72±014b	48.67±043	85.72±1.01

There are data showing that the element release rate (i.e. Al, Cu, Fe, K, Mg, Mn, Na, Ni, Zn) is increasing with increasing infusion time. The element concentrations level in tisanes of herbal and medicinal tea formulations is strongly affected by a number of parameters, including the composition of given teas and formulations, their original element content and pH of water used for tisanes preparation as well as solubility of minerals and other matrix components such as phenolic compounds. Decreased concentrations of some elements (i.e. Al, Cu, Fe, Mn) in the herbal brews can also be a result of the formation of their insoluble complexes with organic substances or their hydrolysis and precipitation [24].

Bioaccessibility can be defined as the amount of a food constituent that is available in the gut, as a consequence of the release of this constituent from the food media and may be able to run through the intestines. Only polyphenols released from the food media by the action of digestive enzymes (small intestine) and bacterial micro-flora (large intestine) are bioaccessible in the gut and for this reason potentially bioavailable [25]. Antioxidant capacity results of the beverage are shown in Table III. Total phenolic content of bioaccessible phenolics of dried linden leaves and blossoms and MLTB were analysed respectively as 1507.59±19.25 mg GAE/100 g and 312.82±5.91 mg GAE*/100 mL.

TABLE III
THE ANTIOXIDANT CAPACITY RESULTS OF MINERAL ENRICHED LINDEN HERBAL TEA BEVERAGE (MLTB)

	Antioxidant capacity	
	Chemical extract (µmol trolox/mL)	Physiological extract (µmol trolox/mL)
DPPH	27.59±0.53	0.17±0.02
FRAP	21.01±0.97	13.27±0.19
CUPRAC	44.71±9.42	2.80±0.64

Phenolic content and antioxidant activity of linden herbal tea beverage were determined as lower than dried linden, as a result of the production process of herbal tea beverage in which 1% of herbal extract was used and thermal treatment was applied. In comparison with antioxidant capacity essays, DPPH had the highest value in chemical extract of linden herbal tea beverage. However, FRAP assay had the highest value in physiological extract.

IV. CONCLUSION

As a result of the growing awareness about healthy food consumption, functional beverages gained importance worldwide. Accordingly, linden herbal tea beverage enriched with natural mineral water was preferred by the panelists in our study. Consequently, it is thought that, this ready-to-drink and thirst quenching beverage will satisfy the consumer's demand for nutritious, healthy soft drinks and thus may have good potential for commercialization.

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