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Bioacessibility of phenolic compounds and antioxidant capacity in organic peppermint leaves

Bioaccesibilidad de compuestos fenólicos y capacidad antioxidante en hojas de menta de pimienta orgánica

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ABSTRACT

The objective of this study was to analyze the chemical composition and bioaccessibility of phenolic compounds as well as their antioxidant capabilities of organic peppermint leaves after each phase of simulated digestion. Moisture was determined until a constant weight was obtained in an oven at 105 °C; ash was determined after sample calcination in a muffle furnace at 550 °C. The protein concentration was determined by the Macro-Kjeldahl method and lipid content by hot-extraction in a Soxhlet apparatus. Carbohydrates were calculated from differences and energy values based on the Atwater conversion factors. Total phenolic, flavonoid content, and antioxidant activity were determined by spectrophotometry. A four-step procedure was used for in vitro digestion. Organic peppermint was found to contain the following: 78% moisture, 1.7% ash, 1.5% lipids, 0.3% proteins, 17.7% carbohydrates, and a total of 85.5 kcal/100 g. Values of 705 mg GAE/100 g of phenolic, 918 mg QE/100 g of flavonoids, and 58.8 mg/g of vitamin C were also measured. It was discovered that total phenolics had the highest bioaccessible fraction relative to flavonoids; the salivary phase was identified as that with the highest release of these compounds and thus the phase in which peppermint showed significant antioxidant activity (1509 µmol TEAC/100g). This study demonstrated that organic peppermint has a high content of phenolic compounds that can be extracted from the alimentary matrix in the salivary and intestinal phases of the digestive system. Because of the antioxidant activity of these compounds, the use of this aromatic plant as seasonings and spices is relevant.

Keywords: Antioxidant capacity; Bioactive compounds; Chemical composition; Mentha piperita L.; Simulated digestion.

RESUMEN

El objetivo de este estudio fue analizar la composición química de hojas de menta orgánica y la bioaccesibilidad de los compuestos fenólicos así como su capacidad antioxidante. Se determinó la humedad hasta obtener un peso constante en el horno a 105 °C, la ceniza se determinó en un horno de mufla a 550 °C. La concentración de proteína se determinó mediante el método Macro-Kjeldahl y los lípidos mediante extracción en un aparato Soxhlet. Los carbohidratos se calcularon por diferencia y energía basadas en los factores de conversión de Atwater. El contenido fenólico y la actividad antioxidante se determinaron por espectrofotometría. Se usó un procedimiento de cuatro pasos para la digestión in vitro. La menta orgánica contiene 78% de humedad, 1,7% de cenizas, 1,5% de lípidos, 0,3% de proteínas, 17,7% de carbohidratos y 85,5 kcal/100 g. También se midieron valores de 705 mg GAE/100g de fenólico, 918 mg QE/100 g de flavonoides y 58,8 mg/g de vitamina C. Se descubrió que los fenólicos tenían la fracción bioaccesible más alta en relación con los flavonoides; la fase salival se identificó como aquella con la mayor liberación de estos compuestos y, por lo tanto, la menta mostró una actividad antioxidante significativa (1509 µmol de TEAC/100 g). La menta orgánica tiene un alto contenido de fenólicos que se pueden extraer en las fases salival e intestinal. Debido a la actividad antioxidante de estos compuestos, el uso de esta planta como condimentos y especias es relevante.

Palabras clave: Capacidad antioxidante; Compuestos bioactivos; Composición química; Digestión simulada; Mentha piperita L.

INTRODUCTION

The search for food using more sustainable production systems such as organic production methods has increased worldwide. Agro-ecological systems are beneficial because they reflect nutritional value, concerns related to food production and conservation processes, and the environment. Although studies of the nutrient contents of organic foods are inconclusive, organic system have been shown to be effective for reducing chemical additives that may enter the food chain[1].

Aromatic and medicinal plants have gained attention for their important roles in health, foods, and essences. The chemical, pharmacological, food, and cosmetic industries use raw materials such as members of the Lamiaceae family that includes peppermint (Mentha piperita L.). The cultivation of these species has economic potential[2].

Several studies have reported the presence of a wide variety of compounds such as terpenoids, iridoids, flavonoids, and phenolic compounds in plants of the Lamiaceae family[3,4]. Another component of peppermint is a volatile oil composed mainly of menthol, menthone, menthofuran, and menthyl acetate. Other pharmacologically active ingredients comprise bitter substances, caffeic acid, flavonoids, polymerized polyphenols, carotenes, tocopherols, betaine, choline, and tannins[5].

These phytochemicals can help reduce the oxidative damage associated with several diseases, including cancer, cardiovascular diseases, atherosclerosis, diabetes, and arthritis, among others[6]. When consumed as food, it is important to note that humans do not effectively utilize all ingested compounds. During the digestive process, these compounds undergo biotransformation. Thus, the bioaccessible fraction of a compound can be understood as the amount of the compound released from its matrix into the gastrointestinal tract and available for absorption[7].

This study was conducted to analyze the chemical composition and bioaccessibility of phenolic compounds, as well as antioxidant capacity, after each phase of simulated digestion (*in vitro*) of organic peppermint (Mentha piperita L.) leaves.

MATERIALS AND METHODS

Location and study period

This study was conducted in the Laboratory of Bromatology and Biochemistry of Food/Antioxidants and Liquid Chromatography Room of the Department of Nutrition of the Federal University of Piauí, from September 2016 to February 2019.

Raw materials

The organic peppermint samples were purchased in an establishment specialized in organic product sales in Teresina-PI. The presence of the SISORG (Brazilian Organic Conformity Assessment System) seal was used as the selection criterion.

Centesimal composition

The moisture content of the samples was determined after obtaining a constant weight after drying in an oven at 105 °C. Ash content was determined after calcination of the samples in a muffle furnace at 550 °C. Protein concentration was determined using the Macro-Kjeldahl method with a conversion factor of 5.75. Lipid content was measured by hot intermittent extraction using hexane as a solvent in a Soxhlet apparatus[8]. Carbohydrate content was calculated as a difference (carbohydrates= 100 - (moisture+ash+protein+lipids) and total energy value according to Atwater conversion factors[9].

Vitamin C

Tillman's method, which is based on the reduction of 2,6-dichlorophenolindophenol sodium salt dye by an acid solution of vitamin C, was used to determine vitamin C content[8].

Phenolic compounds

Several reagents were tested for preparing the extracts, as the solubility of phenolic compounds varies as a function of solvent polarity, degree of polymerization of the compounds, and their interactions.

To obtain the extracts, samples went through a pre-drying process in a ventilated oven (60 °C/2h). The highest extraction yields of peppermint were obtained when 50% methanol: 70% acetone:water solution (2:2:1) was used[10].

The contents of bioactive compounds were measured by spectrophotometry. Total phenolics[11] and total flavonoids[12,13].

Antioxidant capacity - 2,2 - diphenyl-1- picrylhydrazyl (DPPH) method

The scavenger effect of DPPH radicals in the sample was determined by spectrophotometry[14]. Results were expressed as µmol TEAC (trolox equivalent antioxidant capacity)/100 g of the sample.

Bioaccessibility

Bioaccessible fractions of antioxidant compounds were estimated using a four-step procedure (salivary, gastric, intestinal, and colonic), in which the human digestive process was simulated *in vitro* using synthetic digestive fluids[15,16].

Statistics

Statistical analyses of the data were performed using SPSS version 21.0 software (SPSS, Inc., Chicago, IL, USA). For multiple comparisons, the Tukey test was used at a 5% significance level and the 95% confidence interval[17].

RESULTS

Table 1 presents proximate composition, total energy value, content of phenolic compounds, and antioxidant capacity of organic peppermint leaves. Average values of three repetitions and the standard deviation are shown.

Table 2 shows phenolic compound content and antioxidant capacity of peppermint leaves (Mentha piperita L.) after each simulated digestion phase. High antioxidant capacity was verified in the salivary phase.

Table 3 shows the result of in vitro bioaccessibility of phenolic compounds and antioxidant capacity of organic peppermint leaves. A statistically significant difference was observed between phenolic compounds ($p \leq 0.05$).

DISCUSSION

The chemical composition of the organic peppermint leaf is shown in table 1. These samples had a high moisture content (78.8 g/100 g). Research that analyzed plants in the Lamiaceae family (rosemary and basil) also found high moisture contents of 63.6 and 82.7 g/100 g, respectively. To increase the shelf life of these foods, they must be dried to reduce high moisture content[18,19].

The ash content was found to be 1.7 g/100 g (Table 1). In a study of medicinal plants sold in Cuiabá (MT), ash levels ranged from 1.6 to 8.4 g/100 g. Contents obtained in the present study agree with those of the previous study with acceptable variation, as the raw materials and cultivation conditions used were different[20].

As shown in table 1, among macronutrients, the carbohydrate content was 17.7 g/100 g and protein levels were low (0.32 g/100 g). A study that analyzed samples of rosemary pepper, French basil, eucalyptus, and elderberry found lipid contents of 1.04, 1.44, 2.33, and 6.26 g/100 g, respectively, which are comparable to the value obtained

Table 1. Chemical composition and total energetic value (TEV) of organic peppermint.

Analysis	Peppermint (mean ± standard deviation)
Moisture (g/100g)	79±0
Ash (g/100g)	1.7±0
Lipids (g/100g)	1.5±0
Proteins (g/100g)	0.3±0
Total carbohydrates (g/100g)	17.7
TEV (Kcal/100g)	85.5
Vitamin C (mg/g)	59±0
Total phenolics (mg GAE/100g)*	705±5
Total flavonoids (mg QE/100g)**	918±5
Antioxidant capacity (µmol TEAC/100g)***	4431±3

* GAE – Equivalent to gallic acid.
** QE – Equivalent to quercetine.
*** TEAC – Antioxidant capacity equivalent to trolox.

Table 2. Phenolic compounds and antioxidant capacity of peppermint leaf (Mentha piperita L.) after each simulated digestion phase.

Phase	Total phenolics (mg GAE/100g) Mean ± SD	Flavonoids (mg QE/100g) Mean ± SD	Antioxidant Capacity DPPH (µmol TEAC/100g) Mean ± SD
Salivary	3711±12[a]	364±10[a]	1509±0[b]
Gastric	143±5[a]	101±16[b]	438±0[c]
Intestinal	362±6[a]	400±6[b]	361±4[c]
Colonic	100±0[a]	58±2[b]	393±9[c]

Different subscribed letters, on the same line, represents a significant difference between means according to One Way ANOVA: Post Hoc Multiple Comparisons test, the Tukey's test was used at the level of 5% p<0.05, 95% CI.

Table 3. Bioaccessible fraction of phenolic compounds and antioxidant capacity of peppermint leaf (*Mentha piperita* L.) after each simulated digestion phase.

Phase	Total phenolics (mg GAE/100g^{-1}) %	Flavonoids (mg QE/100g^{-1}) %	Antioxidant Capacity DPPH (µmol TEAC/100g) %
Salivary	53[a]	40[b]	34[c]
Gastric	20[a]	11[b]	10[c]
Intestinal	51[a]	43[b]	8[c]
Colonic	14[a]	6[b]	9[b]

Different subscribed letters, on the same line, represents a significant difference between means according to One Way ANOVA: Post Hoc Multiple Comparisons test, the Tukey's test was used at the level of 5% p<0.05, 95% CI.

in the present study (1.50 g/100 g)[18]. Several researches have reported that plants contain essential oils with diverse functions, including therapeutic properties.

Components with significant antioxidant effects also are detected in foods, which may be useful for preventing diseases. Vitamin C content in peppermint leaves was 59 mg/g. Vitamin C levels of 126.63 mg/100g were found in peppermint (dry weight). These results reflect the differences in the chemical composition of leaves due to the edaphoclimatic conditions[21].

A study determined the total phenolic content in six species of wild Mentha (*Lamiaceae*) from Northeastern Algeria[22]. The levels obtained varied from 1466 to 4321 mg GAE (gallic acid equivalent)/100 g, dry weight, which are higher than levels obtained in the present study (705 mg GAE/100 g). This may be because of genetic variation, harvesting season, climatic conditions, soil, cultivation method, stage of maturation, and other factors. The solvent used to prepare the extracts is also important; in this study, used 50% methanol: 70% acetone:water solution, while the reported research used 80% methanol.

Among the phenolic compounds, flavonoids were prevalent. Peppermint leaves showed a flavonoid content of 918 mg quercetin equivalent/100 g (Table 1). A research study evaluated spices commonly consumed in southern Nigeria and found total flavonoid content of 12–147 mg

QE/100 g. Therefore, peppermint has a higher flavonoid content than *Aframomum citratum, Afrostyrax lepidophyllum, Ricinodendron heudelotii,* and *Monodora myristica*[23].

Given the phenolic results, it is noteworthy that the extraction of phenolic compounds is performed with the use of organic solvents such as methanol, ethanol, acetone, water, ethyl acetate, propanol, dimethylformaldehyde and their combinations. Therefore, the solubility of phenolics varies according to the polarity of the solvent used, the degree of polymerization of the compounds and their interactions with other constituents of food, such as carbohydrates and proteins. These interactions are common, since food is a complex matrix, and together with the solvents used, they may have underestimated the total phenolic content obtained in peppermint leaves[24]. Similar results have been obtained in other studies.

In a study that analyzed *Teucrium polium* extracts, levels of flavonoids higher than those of total phenolics were found in three types of extracts (methanolic, water, and ethyl acetate). It should be noted that, in extracts made with water and ethyl acetate, flavonoid content was twice as high as those determined for total phenolics. In turn, extract made with ethanol obtained a lower flavonoid content than that obtained from total phenolics[25].

A similar result was also reported in a study of *Tectaria paradoxa* (Fee.) Sledge, where the total flavonoid contents of 1384; 1104; 707 and 1096 mg QE/g were determined in extracts made with methanol, chloroform, petroleum ether and acetone, respectively. In turn, these extracts obtained lower levels of total phenolics (351; 335; 289 and 333 mg GAE/g, respectively)[26].

The highest antioxidant capacity of peppermint was observed in the salivary phase (1509 µmol TEAC/100 g) (Table 2). The results of antioxidant capacity measurements were consistent with the bioaccessible fraction of these compounds throughout simulated digestion.

Because these bioactive compounds are affected by the digestive process, table 3 shows the results of bioaccessibility measurement of the phenolic compounds after each phase of simulated digestion. Total phenolics showed higher bioaccessible fractions compared to total flavonoids in all phases of simulated digestion; therefore, total phenolics are released from the food matrix in the gastrointestinal tract in greater quantities ($p \leq 0.05$).

In simulated digestion, the salivary and intestinal phases had the highest bioaccessibility for phenolics and flavonoids; thus, the salivary phase appears to be the main site at which phenolic / flavonoid compounds are extracted during in vitro digestion. Analysis of the antioxidant activity of peppermint after each phase of simulated digestion confirmed this result (Table 3).

CONCLUSIONS

Organic peppermint leaves were characterized by high moisture and carbohydrate content. Leaves also contained a high content of total phenolics, total flavonoids, and vitamin C.

Bioaccessibility analysis showed that phenolics had a higher bioaccessible fraction compared to flavonoids, and that the highest release of these compounds occurred during the salivary phase. Thus, peppermint showed the significant antioxidant capacity in this phase.

Organic peppermint contains high levels of phenolic compounds that showed the highest levels of extraction from the alimentary matrix during the salivary phase in the digestive system. Because of the antioxidant capacity of these compounds, the use of peppermint leaves as seasonings and spices, in addition to contributing with sensory aspects, can help in the prevention of chronic diseases and in food conservation. These materials are also important for producing flours for use in enriched food products.

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