

Improvement of bioavailability of bioactive compounds of medicinal herbs by drying and fermentation with *Lactobacillus plantarum*

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ABSTRACT:

Background: Medicinal and aromatic plants, which are rich sources of bioactive compounds, have been used in traditional medicine for ancient times. Epidemiological studies have shown that bioactive compounds of medicinal plants possess antioxidant, anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities. Intake of natural antioxidants derived from medicinal plants into body has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases. It has also been demonstrated that the fermentation of plants is a versatile way for improvement of their functionality and bioavailability. The aim of this study was to determine the effect of probiotic fermentation and vacuum drying on the bioavailability of peppermint (*Mentha piperita*) and basil (*Ocimum basilicum*) exposed to *in vitro* digestion.

Methods: Fresh basil and peppermint were divided to 3 lots, one lot of each plant was vacuum dried at 40 °C for 8 hours to obtain the dried samples while one lot was fermented with probiotic *Lactobacillus plantarum* for 21 days at room temperature after incorporation with sterile brine (1:20 w:v) containing 5 % of NaCl. Thereby, 3 samples were obtained: Fresh peppermint/basil, vacuum dried peppermint/basil and fermented peppermint/basil. During the *in vitro* digestion comprised from gastric and pancreatic digestion, bioavailability of the samples were investigated by measurement of their total phenolic contents (TPCs), total flavonoid contents (TFCs) and antiradical activity. TPC was determined using Folin-Ciocalteu method while TFC was measured aluminum chloride colorimetric assay. The antiradical activity of the samples was analyzed by determination of the DPPH radical scavenging activity.

Results: The initial (undigested) TPC levels of fresh, vacuum dried and fermented mint and basil ranged from 166.24 to 295.08 mg gallic acid equivalent (GAE)/g. Drying and fermentation increased TPCs of the plants. Similarly, TFCs and antiradical activities were also increased by

these treatments. When considering bioavailability of the bioactive compounds, fermentation process enabled higher recovery levels after *in vitro* digestion while basil exhibited higher percent recovery than mint.

Conclusion: This study demonstrated that vacuum drying and fermentation of basil and mint with probiotic *Lactobacillus plantarum* provided much stronger antioxidant activity and bioavailability than fresh ones and increased their *in vitro* bioavailability.

BACKGROUND

Turkey, owing to its outstanding geographical area, hosts very different climatic regions and properties, a rich plant diversity, and includes many endemic species. Although several selected aromatic and medicinal plants are cultivated, a considerable part of them are still widely grown. The use of aromatic and medicinal plants belongs to ancient times for major treatment of diseases in traditional medicine. Other fields of their use has been found in food preservation, food flavoring and cosmetics [1]. Increasing the awareness of consumers about the importance of natural products that contain less food additives has also influenced the use of aromatic plants in their daily life [2]. According to the World Health Organization (WHO), the majority of the populations in developing countries up to 80% use medicinal plants as remedies [3]. In recent years, many research has been focused on revealing medicinal and therapeutical properties of aromatic plants and development of novel drugs [4]. Today, approximately 25% of modern medicines are prepared from natural sources including plants [5]. However, only 15% of the world plant sources has been evaluated for their therapeutical and medicinal potential [6].

Medicinal properties of aromatic plants are attributed to the presence of bioactive compounds, especially phenolics and flavonoids. Phenolics, which can be located in different parts of plants, constitute the most important group of antioxidant compounds [7]. These compounds are generally secondary metabolites which forms defense mechanisms of plants and play several other physiological roles [8]. The intake of natural antioxidants derived from medicinal plant has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing [9, 10].

Basil (*Ocimum basilicum*) and peppermint (*Mentha piperita*), both belonging to family Lamiaceae (Labiatae), are among the most commonly known medicinal plants in the World. Although basil is endemic to Asia, Africa, South America and Mediterranean region, it is cultivated in many countries. Meanwhile, peppermint is more commonly grown in temperate regions including Eurasia, Australia and South Africa [11-14]. Research has shown that both basil and peppermint are rich in antioxidant compounds such as phenolics and possess strong therapeutical and medicinal properties [15].

Fermentation is a traditional and ancient method which change physicochemical, sensorial and nutritional properties of foods with the aim of microorganisms [16]. Fermentative microorganisms modify plant composition by releasing the chemically bound compounds, breaking down plant macromolecules and synthesizing new metabolites. Thereby, fermented plants are enriched by phytochemicals, have improved bioactivity and bioavailability as well as better textural and sensorial properties [17].

To the best of our knowledge, there has been no work focused on the change in the bioavailability of the bioactive compounds of peppermint and basil by vacuum drying and lactic acid fermentation in the literature. The effect of *in vitro* digestion on the bioavailability of these compounds has also not been studied before. Therefore, the aim of this study was to investigate

the effect of vacuum drying and fermentation on bioavailability of antioxidant and bioactive compounds of basil and peppermint treated to *in vitro* digestion.

METHODS

Plant materials and sample preparation

Fresh basil (*Ocimum basilicum*) and peppermint (*Mentha piperita*) were provided from Zeytinburnu Medicinal and Aromatic Plant Garden (Istanbul, Turkey). Well-grown, fresh and mold-free leaves of the plants were selected. Each plant material was divided into 3 lots. One lot was left as fresh and used as the control sample. One lot of the plant was vacuum dried at 40 °C for 8 h using a vacuum oven (Daihan WOV-30, Gangwon-do, Korea) and oil vacuum pump (EVP, 2XZ-2C, Zhejiang, China) with 60 mbar ultimate pressure and 2 L/s pump speed [18]. The remaining sample was fermented with probiotic *Lactobacillus plantarum* uruma SU5 which was isolated from traditionally fermented salgam juice and identified by genotypic characterization of the bacterial genomic DNA by PCR (polymerase chain reaction) technique. For this purpose, the cryopreserved strain was twice activated at 37 °C for 24 hours under anaerobic conditions using de Man, Rogosa and Sharpe (MRS) Broth (Merck, Germany) [19]. Following incorporation of 25 g of the plant material with 500 mL of the sterile brine (containing 5% of NaCl w:v) in a glass jar with metal lid, the brine solution was inoculated with fresh *L. plantarum* (1% v:v). Then it was fermented for 21 days at ambient conditions. After the fermentation, the fermented plant material was removed from the brine solution and used in the experiments immediately.

Determination of lactic acid bacteria population

The population of total lactic acid bacteria of the fermented peppermint and basil samples was analysed using spread plate method, in order to have information about the adaptation of *L. plantarum* to the plant media. For this purpose, 10 g of the sample was incorporated with 90 mL of sterile peptone water. Then the decimal dilutions were prepared. The appropriate dilutions (0.1 mL) were spread plated onto MRS Agar (Merck, Germany) and the plates were incubated at 37 °C for 24 hours at anaerobic conditions. After the incubation, the formed colonies were counted and recorded as colony forming unit per gram. The results were then converted to logarithmic values.

Extraction procedure

In order to determine the antioxidant properties of the plant samples and the change in the bioavailability of antioxidants during *in vitro* digestion, the extracts of the fresh, vacuum dried and fermented basil and peppermint samples were obtained. The samples were incorporated with 80% aqueous ethanolic solution (1:20 w:v) and treated to extraction at room temperature for 4 hours. After removing the plant material, the solution was filtered using Whatman No.4 filter paper to remove the impurities and the solvent was removed using a rotary evaporator. The extract was then dried using a lyophilizer and the powdered extracts were obtained.

In vitro gastrointestinal digestion

Gastric and intestinal digestion procedure was performed by the stimulated *in vitro* digestion model described by McDougall et al. [20] with some modifications, as summarized in Figure 1.

The first step was the gastric digestion. The sample extracts were homogeneously mixed in 20 mL of distilled water and 1.5 mL of the pepsin solution (40 mg/ml) prepared with 0.1 M of HCl.

Then the pH value was adjusted to 2.0 using 1 M of HCl. After lidding the beaker, the mixture was incubated at 37 °C for 2 hours in a shaking incubator with continuous stirring at 100 rpm. At the end of the incubation, 5 mL of PG (post-gastric) fraction was separated and stored at -20 °C. For intestinal digestion, 5 mL of pancreatin (18 mg/mL), a mixture of bile (112.5 mg/mL bile salt) and 4.5 mL of NaHCO₃ (0.1 M) were incorporated with the stomach digested beaker. The dialysis tube was completely immersed in the PG phase and the beaker was sealed with parafilm. Then the mixture was incubated at 37°C for 2 hours in a shaking incubator with continuous stirring at 100 rpm. At the end of intestinal digestion, the fraction in the dialysis tube "IN" in the dialysis tube was transferred into a test tube while the mixture outside the tube was transferred into another test tube as OUT. Here, OUT sample represented the material which remained in the gastrointestinal tract while the IN sample meant the material that entered the serum.

The recovery (%) of bioavailability in the bioactive compounds of the samples were determined using the following equation:

$$\text{Recovery (\%)} = \frac{\text{The activity of IN phase}}{\text{The activity of the undigested sample}} \times 100 \quad (1)$$

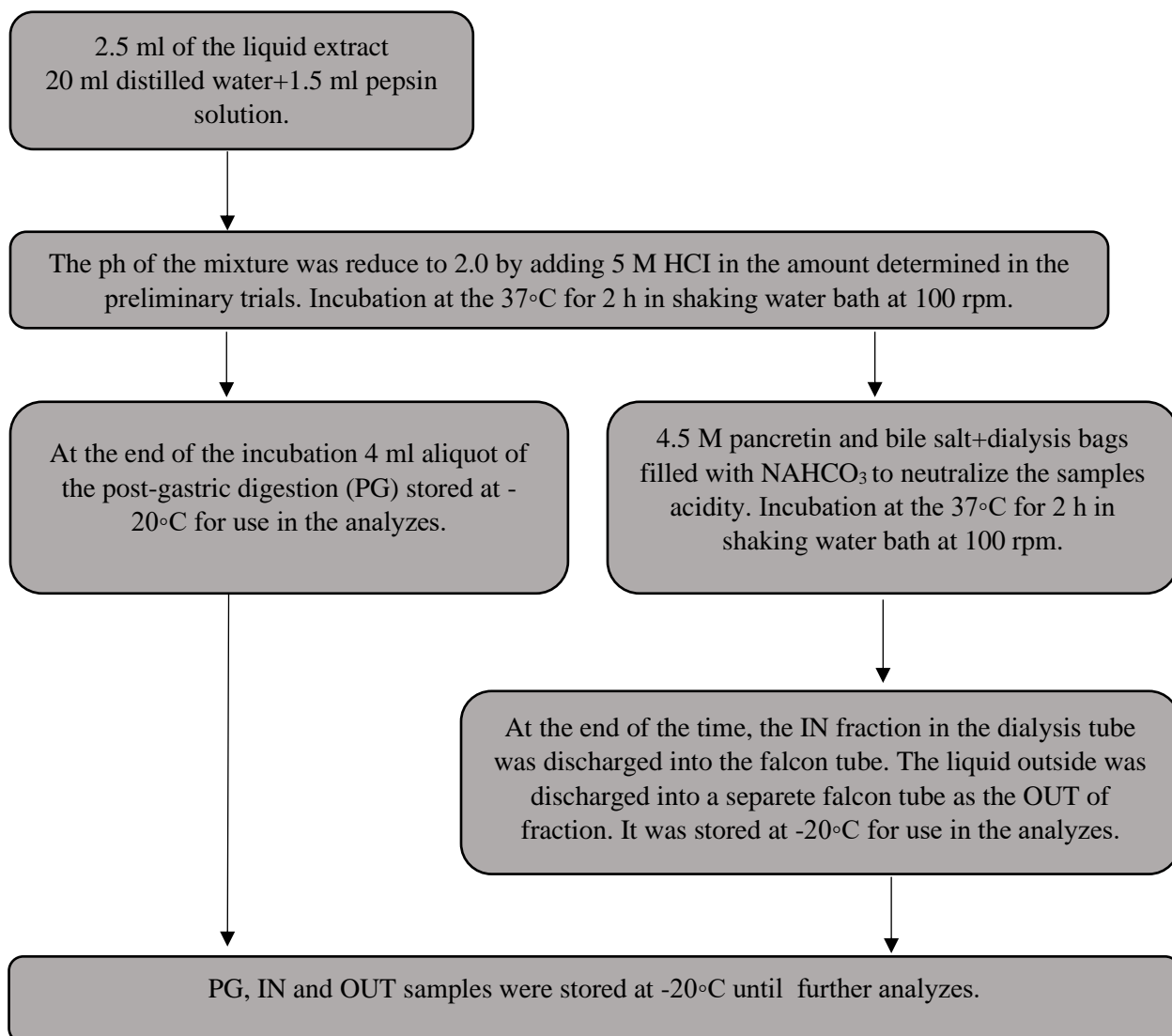


Figure 1. Flow chart of the *in vitro* gastrointestinal digestion method.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the samples was determined using Folin-Ciocalteu method as described by Singleton and Rossi [21]. For this purpose, 0.5 mL of the lyophilized ethanolic extract of the sample was incorporated with 2.5 mL of 0.2 N Folin-Ciocalteu's reagent and incubated for 3 minutes at ambient conditions. Following the incubation, 2 mL of 7.5% Na₂CO₃ solution was added and the mixture was incubated at the room temperature for 30 minutes. Then the absorbance of the samples was measured at 760 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were calculated using the following equation and were expressed as mg gallic acid equivalents of g of the dried extract (mg GAE/g):

$$TPC(\text{mg GAE/g}) = \frac{\text{absorbance} - 0.0791}{0.0103} \times \text{dilution factor} \quad (2)$$

The calibration curve was plotted and the equation (Eq. 2) was found based on the slope of the curve. A linear calibration curve graph was prepared by reading the absorbance of the gallic acid standard at different concentrations using the spectrophotometer. This equation was $y = ax + b$ in the equation $x = (y - b) / a$ total phenolic content was determined. In this equation, $R^2 = 0.999$ and the calibration equation was $y = 0.0103x + 0.0791$, which was used for calculation of the total phenolic content.

Determination of total flavonoid content (TFC)

Total flavonoid content (TFCs) of the fresh, vacuum dried and fermented basil and peppermint samples were determined according to the method described by Zhinsen et al. [22]. For this purpose, 1 mL of extract was mixed with 4 mL of distilled water and 0.3 mL of 5% NaNO₂ solution (w/v). After 5 minutes, it was incorporated with 0.3 mL of 10% AlCl₃ solution (w/v) and mixed for 6 minutes. Following the addition of 2 mL of 1 M NaOH, the volume was completed to 10 mL with distilled water. Then the absorbance values of the samples were measured at 510 nm with UV-VIS spectrophotometer (Shimadzu UV-1800, Japan). All the results were expressed as mg catechin equivalents (CAE)/100 g of the dried extract. The formula used to calculate the total flavonoid content was given in Eq. 3:

$$TFC \left(\text{mg} \frac{\text{CAE}}{\text{g}} \right) = [(151.6 \times \text{absorbance}) - 0.05454] \times \text{dilution factor} \quad (3)$$

Determination of antiradical activity

The antiradical activity of the fresh, vacuum dried and fermented basil and peppermint samples was determined by measurement of DPPH radical scavenging ability. Then 0.1 mL of the sample extract was incorporated with 4.9 mL of 0.1 mM DPPH solution (in ethanol) and the mixture was incubated at 27 °C for 20 minutes at dark conditions. Following the incubation, the absorbance of the samples was measured at 517 nm using a UV-vis spectrometer (Shimadzu, UV-1800, Japan). The results were presented as mg Trolox equivalent (TEAC)/100 g of the dried extract [23].

Statistical analysis

The data is an average of 3 replicates and 3 parallels for each replicate. The average values and standard deviations were calculated using Microsoft Excel software (Office 2016, Microsoft). The average values were compared using SPSS statistical software (SPSS version 20.0, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and Duncan's multiple comparison tests with α set at 0.05 was applied to compare the average values at the 95% confidence level.

RESULTS

Bioactive properties of the extracts

Lactic acid bacteria (LAB) counts of the fermented peppermint and basil was analyzed before extraction procedure. Peppermint and basil samples had 8.48 ± 0.08 and 8.07 ± 0.06 log cfu/g LAB populations. The LAB count of peppermint was significantly higher ($p < 0.05$) than basil.

Following the extraction process (the extraction yield was 7.82% on dry basis) antioxidant properties of the extracts were evaluated. The results are presented in Table 1. Vacuum dried extracts had the highest ($p < 0.05$) TPC and TFC values among the samples. Considering the radical scavenging activity, fermented samples exhibited the highest ($p < 0.05$) values. Overall, the basil exhibited better antioxidant properties ($p < 0.05$) than the peppermint. The fermentation process also increased the antioxidant properties of the basil and peppermint samples at significant levels ($p < 0.05$).

Table 1. Bioactive properties (dry basis) of basil and peppermint extracts.

Sample	TPC (mg GAE/g)	TFC mg CAE/g)	DPPH (mg TE/g)
Fresh peppermint	166.24 ± 2.01^{Cf}	65.53 ± 0.67^{Af}	96.41 ± 0.24^{Bf}
Dried peppermint	286.08 ± 5.56^{Cb}	101.56 ± 1.98^{Ac}	123.47 ± 3.05^{Bd}
Fermented peppermint	231.14 ± 2.11^{Cd}	78.45 ± 1.02^{Ae}	191.60 ± 4.13^{Bb}
Fresh basil	198.35 ± 3.67^{Ce}	95.56 ± 1.42^{Ad}	112.45 ± 1.71^{Be}
Dried basil	295.73 ± 5.45^{Ca}	147.35 ± 2.12^{Aa}	153.21 ± 1.18^{Bc}
Fermented basil	278.87 ± 3.56^{Cc}	129.87 ± 1.37^{Ab}	236.56 ± 2.28^{Ba}

TPC: Total phenolic content; TFC: Total flavonoid content; ^{a-f}: Significant difference between the data on the same column ($p < 0.05$); ^{A-C}: Significant difference between the data on the same line ($p < 0.05$).

In vitro bioavailability of total phenolics

Bioavailability of the phenolics of fresh, dried and fermented peppermint/basil during *in vitro* digestion was analyzed. The results are presented in Table 2. Initial (undigested) TPCs of the samples ranged from 166.24 to 295.73 mg GAE/g. The drying and fermentation process significantly ($p < 0.05$) increased TPCs while vacuum dried samples had the highest results. During the gastric digestion, dramatic decreases were observed.

Table 2. Change in bioavailability of total phenolic compounds of peppermint and basil during *in vitro* digestion.

Samples	TPC (mg GAE/g)			
	Initial	Post gastric (Pg)	Intestinal digestion	
			In	Out
Fresh peppermint	166.24±3.42 ^{Df}	30.61±0.45 ^{Bd}	31.35±1.01 ^{Bdc}	40.59±0.65 ^{Cdc}
Dried peppermint	286.08±3.01 ^{Db}	27.94±0.32 ^{Be}	30.78±1.01 ^{Bd}	38.77±0.42 ^{Cd}
Fermented peppermint	231.14±3.05 ^{De}	83.74±0.31 ^{Ba}	80.58±2.11 ^{Bb}	91.19±0.84 ^{Cb}
Fresh basil	198.35±2.54 ^{Dd}	32.41±0.44 ^{Bc}	33.28±0.95 ^{Bdc}	40.49±0.53 ^{Cdc}
Dried basil	295.73±3.95 ^{Da}	30.83±0.49 ^{Ad}	35.16±1.36 ^{Bc}	42.34±0.55 ^{Cc}
Fermented basil	278.87±2.62 ^{Dc}	81.81±0.45 ^{Ab}	93.90±2.23 ^{Ba}	105.38±1.88 ^{Ca}

TPC: Total phenolic content; ^{a-f}: Significant difference between the data on the same column (p<0.05); ^{A-D}: Significant difference between the data on the same line (p<0.05).

Percent recovery of total phenolics of *in vitro* digested fresh, dried and fermented peppermint and basil samples are indicated in Figure 1. Although drying process enhanced TPCs of peppermint and basil, their percent recovery did not increase in significant level (P<0.05) during *in vitro* digestion. However, percent recovery of TPC of both fermented basil and peppermint was significantly (P<0.05) higher than the fresh ones.

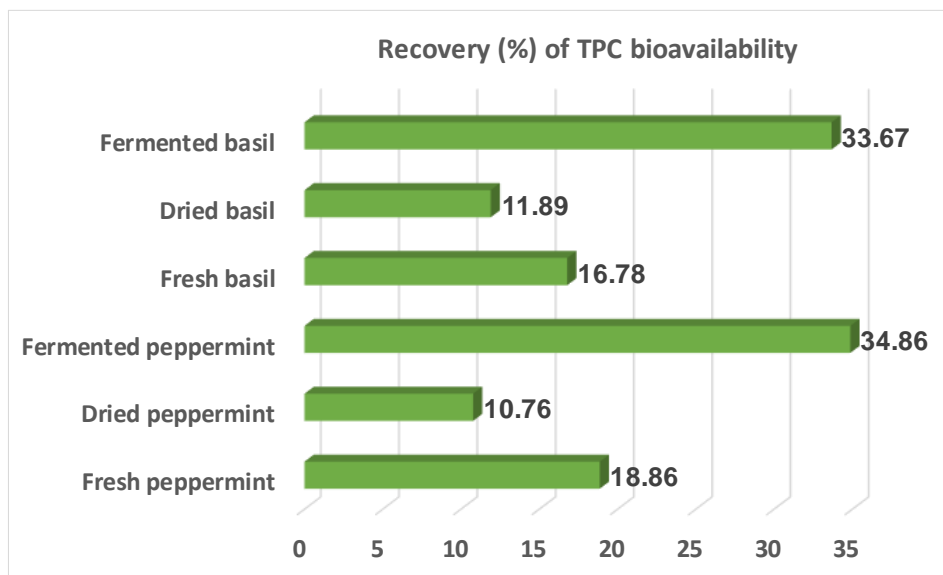


Figure 2. Percent recovery of total phenolics of *in vitro* digested peppermint and basil.

***In vitro* bioavailability of total flavonoids**

Table 3 shows the effect of fermentation and drying on bioavailability of total flavonoids of peppermint and basil. Initial (undigested) TFCs of peppermint and basil ranged from 65.53 to

101.56 and 95.56 to 147.35 mg CAE/g, respectively. Fermentation and drying significantly ($p < 0.05$) increased initial TFC of the samples while TFC of basil was higher ($p < 0.05$) than peppermint. During *in vitro* post gastric digestion, significant ($p < 0.05$) decreases in the bioavailability of the flavonoids of all samples were observed. As similar to phenolics, fermentation enabled the highest ($p < 0.05$) values of the flavonoids for both peppermint and basil. A significant ($p < 0.05$) decrease in TFC was observed in all the samples tested after *in vitro* post-gastric digestion.

Table 3. Change in bioavailability of total flavonoid compounds of peppermint and basil during *in vitro* digestion.

Samples	TFC (mg CAE/g)			
	Initial	Post gastric (Pg)	Intestinal digestion	
			In	Out
Fresh peppermint	65.53±1.16 ^{Df}	5.19±0.1 ^{Bce}	4.90±0.26 ^{Bd}	6.53±0.20 ^{Cf}
Dried peppermint	101.56±1.85 ^{Dc}	6.22±0.19 ^{Bd}	5.31±0.38 ^{Bd}	9.29±0.15 ^{Ce}
Fermented peppermint	78.45±1.70 ^{De}	13.71±0.41 ^{Bb}	14.99±0.52 ^{Bb}	19.87±0.36 ^{Cb}
Fresh basil	95.56±2.79 ^{Dd}	7.48±0.29 ^{Bc}	8.52±0.43 ^{Bc}	13.33±0.22 ^{Cd}
Dried basil	147.35±1.70 ^{Da}	5.70±0.33 ^{Bed}	6.38±0.31 ^{Bd}	16.39±0.23 ^{Cc}
Fermented basil	129.87±2.13 ^{Db}	20.65±0.35 ^{Aa}	28.78±0.32 ^{Ba}	35.12±0.27 ^{Ca}

TFC: Total flavonoid content; ^{a-f}: Significant difference between the data on the same column ($p < 0.05$); ^{A-D}: Significant difference between the data on the same line ($p < 0.05$).

Figure 2 also shows the percent bioavailability (recovery) of the flavonoids after *in vitro* digestion of fresh, fermented or vacuum dried peppermint/basil. It is clear from the figure that the recovery of the fermented peppermint and basil was significantly ($p < 0.05$) higher than the fresh and vacuum dried ones, while the vacuum dried samples had the lowest recovery values. Meanwhile, TFC recovery values were found to be lower than those of phenolics.

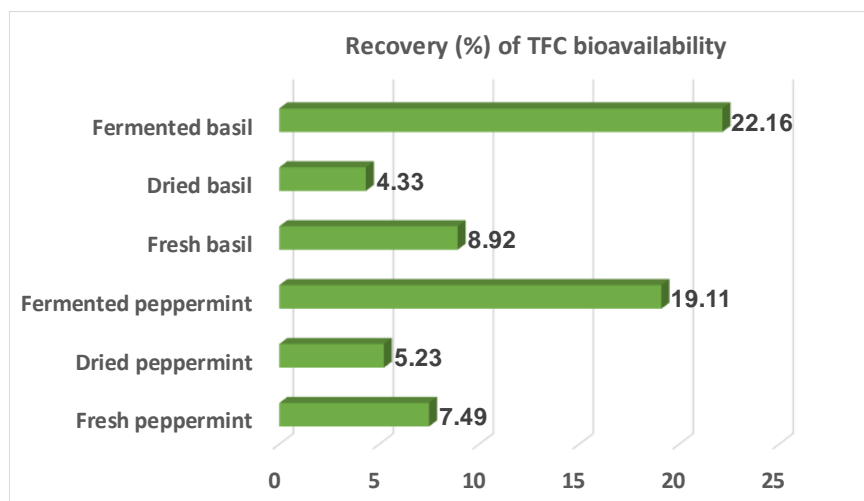


Figure 3. Percent recovery of total flavonoids of *in vitro* digested peppermint and basil.

***In vitro* bioavailability of antiradical activity**

The effect of fermentation and drying on *in vitro* bioavailability of peppermint and basil are presented in Table 4. The fresh, vacuum dried and fermented peppermint and basils had DPPH radical scavenging ability values ranging from 96.41 to 191.60 and from 112.45 to 236.56 mg TEAC/100 g, respectively. Unlike to TPC and TFC, fermentation process enabled higher increase ($p < 0.05$) on antiradical activities of peppermint and basil than drying. Although significant ($p < 0.05$) decreases were seen on antiradical activity values during the *in vitro* gastric and intestinal digestion, fermented basil and peppermint samples exhibited higher ($p < 0.05$) DPPH scavenging activity than vacuum dried and fresh ones. Drying process did not make significant ($p > 0.05$) contribution to antiradical activity of both basil and peppermint.

Table 4. Change in bioavailability of antiradical activity of peppermint and basil during *in vitro* digestion.

Samples	DPPH scavenging activity (mg TEAC/100 g)			
	Initial	Post gastric (Pg)	Intestinal digestion	
			In	Out
Fresh peppermint	96.41±1.45 ^{Df}	30.65±1.61 ^{Be}	32.85±0.66 ^{Bd}	41.31±0.84 ^{Cd}
Dried peppermint	123.47±3.14 ^{Dd}	20.54±1.20 ^{Bf}	23.06±1.10 ^{Be}	29.87±0.36 ^{Ce}
Fermented peppermint	191.60±3.86 ^{Db}	77.10±1.70 ^{Bb}	72.21±2.52 ^{Bb}	82.10±1.33 ^{Cb}
Fresh basil	112.45±2.22 ^{De}	42.94±0.88 ^{Bc}	39.62±1.12 ^{Bc}	50.08±0.80 ^{Cc}
Dried basil	153.21±2.89 ^{Dc}	39.52±0.76 ^{Ad}	42.88±1.49 ^{Bc}	50.45±0.55 ^{Cc}
Fermented basil	236.56±4.97 ^{Da}	90.40±0.98 ^{Aa}	98.20±1.68 ^{Ba}	110.34±1.60 ^{Ca}

^{a-f}: Significant difference between the data on the same column ($p < 0.05$); ^{A-D}: Significant difference between the data on the same line ($p < 0.05$).

Figure 3 presents information about the recovery of antiradical activity of peppermint and basil as a result of *in vitro* digestion. The recovery values of the samples varied from 18.67 % to 41.51 % while basil exhibited higher recovery than basil. It was also clear from the figure that fermentation provided the highest ($p < 0.05$) recovery of DPPH radical scavenging activity.

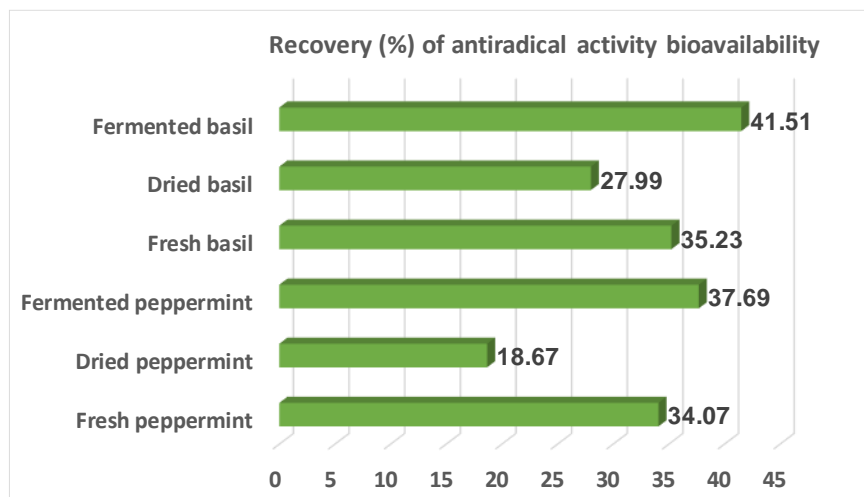


Figure 4. Percent recovery of antiradical activity of *in vitro* digested peppermint and basil.

DISCUSSION

This study was conducted to determine the effect of drying and fermentation processes on bioactive properties of peppermint and basil. This study also aimed to reveal the change in bioavailability of phenolics, flavonoids and antioxidants of the plants during *in vitro* digestion as affected by fermentation and drying.

It is known that plants contain many antioxidant compounds including phenolic compounds, flavonoids, carotenoids and anthocyanins [24, 25]. Antioxidant activity of aromatic plants are mainly attributed to phenolic compounds, which exhibit activities in different ways such as singlet oxygen scavenger, metal chelator, free radical reducing agent or hydrogen donors [26-28].

A number of *in vitro* and *in vivo* test methods have been developed for determination of antioxidant and bioactive properties of foods rich in phenolics [29, 30]. However, since each method measures only a specific reaction, use of multiple methods to determine the antioxidant activity of plants have been proposed [30]. In this study three methods were used, namely TPC, TFC and DPPH radical scavenging activity assays were conducted as indicators for bioactive and antioxidant properties of peppermint and basil and the results were evaluated. In general, there was a positive correlation between the data obtained from different methods, which suggests that the peppermint and basil had high antioxidant activity and bioactive properties. There was a correlation between total amount of phenolic compounds and total amount of flavonoid substances, but not between total amount of phenolic compounds and antioxidant activities. The results were in accordance with the findings in the literature [31-34].

Fermentation is one of the oldest food preservation methods which is still commonly traditionally and industrially used. During fermentation process, a number of biochemical events happen and many changes take place in nutritive and anti-nutritive composition of the raw material [35]. It has been demonstrated that fermentation provided enhanced bioactive properties of different substrates such as legumes, cereals and other plants [36-40]. In the meanwhile, microbial species is one of the most critical factors affecting the change in the bioactive properties of plant based foods during fermentation [26]. *L. plantarum*, one of the lactic acid bacteria species, is widely used for improvement of bioactivity of plants by fermentation [36, 40-42]. This study demonstrated that antioxidant activity and bioactive properties of both peppermint and basil was improved by vacuum drying and fermentation using *L. plantarum* during the *in vitro* digestion period. The results revealed that drying of basil and peppermint increased their bioactive properties due to the being of the bioactive components more concentrated in the sample by drying. Wojdylo et al. [43] reported that heat drying destroyed anthocyanins, flavanols, and ascorbic acid of a strawberry, and there was a significant decrease in antioxidant activity, while vacuum-microwave drying could produce higher-quality products with higher antioxidant activity. Further suggesting that drying method significantly affects bioactive properties of the dehydrated product. Joshi et al. [44] found that concentration of all the phenolic compounds in vacuum- vacuum dried apple slices at 20 °C for 24 hours was comparable to that of fresh apple slices.

The *in vitro* digestion models give opportunity to mimic the physiological conditions of the gastrointestinal tract of humans throughout the transit of food components [45]. At the same time, true studies carried out in animals or human subjects are high-cost, time consuming and very complexed [20]. Therefore, several *in vitro* test procedures has been developed by different researchers [20, 46, 47]. The *in vitro* digestion model which was developed by McDougall et al

[20] allows the screening of multiple samples and may provide data on the relative potential bioavailability of different polyphenolic components.

In the current study, *in vitro* bioavailability of antioxidant and bioactive properties of fresh, vacuum dried and fermented basil and peppermint was evaluated. Although antioxidant properties of the plants decreased during *in vitro* digestion, fermentation enabled better recovery of antioxidant compounds. Bakir et al. [48] found that recovery values of TPC, TFC and antiradical (DPPH scavenging) activity of grape vinegar were 83.6%, 37.1% and 18.5% while they were 73.8%, 100.8% and 23.0% for apple vinegar, respectively. In the meanwhile, recovery of the bioavailability of DPPH scavenging ability of the plants were higher than those of TPC and TFC probably due to the variability of the specific compounds employed in each test and their variable stability at gastrointestinal conditions and interaction with digestive enzymes [49].

CONCLUSIONS

This study revealed that fresh peppermint and basil were rich in antioxidant compounds, especially phenolics and flavonoids. Vacuum drying and fermentation of the plants with probiotic *L. plantarum* increased the antioxidant and bioactive properties, as measured by TPC, TFC and DPPH radical scavenging activity assays. *In vitro* digestion assay showed that gastric and intestinal digestion decreased antioxidant properties of basil and peppermint. However, the fermentation process prevented the loss of bioavailability of the antioxidant during digestion, as determined by higher recovery and IN values. In conclusion, this study confirmed that fermentation acted as a versatile way of protection of plant bioactives and antioxidants against degradation and increase of their bioavailability.

Abbreviations: CAE, catechin equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GAE, gallic acid equivalent; Pg, post gastric; TEAC, Trolox equivalent antioxidant capacity; TFC, total flavonoid content; TPC: total phenolic content.

Competing Interests: The authors declare no conflicts of interest associated with this manuscript.

Authors' Contributions: MSc. Dogan conducted the study and performed the tests. Dr. Tornuk wrote the manuscript, coordinated the study and analyzed the data.

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