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Effects of adding honey at different temperatures to linden tea on antioxidant properties and hydroxymethylfurfural formation

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RESEARCH ARTICLE

Abstract

Linden, a popularly consumed product, is prepared by different methods like infusion and decoction for treating diseases in children and adults. Due to linden's effective compounds and its sweetening, it is used in conjunction with honey and linden tea. This study investigated the effect of adding honey to linden tea prepared by infusion and decoction. Samples held at different temperatures were checked 5, 10, and 15 minutes after filtration to monitor total antioxidant activity, total phenolic compounds, total flavonoid and hydroxymethylfurfural (HMF) levels. Total antioxidant activity, total phenolic compounds and total flavonoids were found to be higher in the sample prepared by decoction. HMF was not observed in the linden tea prepared by infusion. HMF found in the teas prepared through decoction changed from 0.48 to 0.56 µg/ml. The highest concentration of HMF was found in the samples with honey added immediately after filtration (95 °C). The results suggest that when linden tea is prepared, honey should be added at least 10 minutes later to increase total antioxidant activity and to reduce HMF formation.

Keywords: decoction, herbal tea, infusion, phenolic compound, TEAC

1. Introduction

Medicinal plants have been used for many years to treat diseases and protect human health. Secondary metabolites produced by these plants have a positive effect on human health (Ksouri *et al.,* 2012). Secondary metabolites (phenols, flavonoids, tannins, carotenoids, alkaloids, etc.) contained in medical plants are found in a wide range of biological and pharmacological properties. Plants used for phytotherapy are generally prepared as decoction, infusion, tonic, and tincture, according to their properties and are called herbal tea. The therapeutic effects of these herbal teas are associated with antioxidant activity, and phenolic compounds, flavonoids, have been reported to be major contributors to antioxidant capacity (Li *et al.,* 2013). Many studies have shown that herbal tea has a high phenolic content and antioxidant capacity in addition to having positive effects on human health (Li *et al.,* 2013; Lia *et al.,* 2014; Qasima *et al.,* 2017; Skotti *et al.,* 2014). It is used to treat both irritative and dry coughs in the simple inflammation of the respiratory tract by covering the pharyngeal mucosa with its mucilage (Zeybek and Haksel, 2010). Additionally, it is frequently consumed by adults and children for expectorant, inflammatory disease and due to its diaphoretic, diuretic effects in infectious diseases (Toker *et al.,* 2004). Drog can be consumed as an infusion or decoction. The effect is increased when consumed as a decoction (Zeybek and Haksel, 2010). As an integral part of traditional medicine and having nearly 200 different components, honey is sometimes added to tea (Küçük *et al.,* 2007). Rich in antioxidants as well as phenolic acids, flavonoids, carotenoids, organic acids, amino acids, protein, honey also contains Maillard reaction products (Habib *et al.,* 2014). When submitted to heat treatment, honey demonstrates a property known as the Maillard's reaction toxicity, whereby a reduction of sugar and protein interactions causes the formation of hydroxymethylfurfural (HMF) (Kowalski, 2013). High amounts of HMF were reported to have an irritating effect on the upper respiratory tract, membranes of the skin, eyes, and mucous membranes (Ulbricht *et al.,* 1984). Some studies examined the antioxidant capacity by adding honey

to herbal teas (Özdatlı *et al.,* 2014; Pereira *et al.,* 2013). However, no studies that examined the effects of honey added at different temperatures were found in the literature. This study investigated the total antioxidant capacity, total phenolic compounds, total flavonoid values and the effect of different temperatures on the amount of HMF formed in linden tea prepared by infusion and decoction.

2. Materials and methods

Materials

Dried linden flower and flower honey bought from a market in İzmir, Turkey, were used in this study. The study was performed on the basis of consumer habits. Two different linden tea samples were prepared by infusion and decoction. For linden tea prepared by infusion, 2 g of linden were weighed, placed in 240 ml boiling water (98 °C), and filtered after 5 minutes. Honey was not added to one of the five samples of linden tea prepared by infusion, while 7 gram of honey was added to the other four samples. To the second sample immediately after filtration (85 °C), 5 minutes after filtration in the third sample (65 °C), and 10 minutes after filtration in the fourth sample (55 °C). After 15 minutes of filtration (49 °C), samples were prepared for analysis.

For samples prepared by decoction, 2 g of linden was weighed and 240 ml of water (room temperature) was added. The mixture was heated on a hot plate and filtered after boiling. Five different tea samples were prepared, with honey not being added to one sample. The other samples were prepared by adding 7 g of honey. Honey was added to samples, immediately after filtration (95 °C), 5 minutes after filtration (76 °C), 10 minutes after filtration (68 °C) and 15 minutes after filtration (52 °C). The linden tea samples were prepared in duplicate and the samples were analysed.

Total phenolic compounds analysis

To determine total phenolic compounds, the phenolic materials were reacted with a Folin-Ciocalteu solution to form complexes. The resulting colour was measured on a colorimetric basis (Çağındı, 2016; Rodriguez *et al.,* 2015). During the process, samples of 500 µl linden tea mixed with vortex for 30 seconds were taken. 250 µl of 1 N Folin-Ciocalteu reagent was added and mixed with vortex for 30 seconds. This mixture was then thoroughly mixed with the addition of 1.250 µl (20%) of saturated Na_2CO_3 solution. The resulting mixture was held at room temperature for one hour in a dark setting. Subsequently, the resulting colour absorbance was read at 760 nm on a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific, Waltham, MA, USA). The same method was applied to the gallic acid standards to generate a calibration graph ($y = 65.79x + 0.0108$, $R^2=0.998$) and the results were given as gallic acid equivalent (GAE).

Total flavonoid content

Total flavonoid content was determined by a modification of the method by (Rodriguez *et al.,* 2015). 500 µl of linden tea samples were mixed with vortex for 30 seconds. 1.250 µl of distilled water and 75 µl of 5% $NaNO_2$ were then added to the test tube. After 6 minutes, 240 µl of 10% $AlCl_3$ was added, with 500 µl of 1 M NaOH added 5 minutes later. The resulting mixture was read at 510 nm in a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific) after the colour mixture was held at room temperature in a dark setting for 30 minutes. The same method was applied to the prepared catechin standards and the calibration curve was drawn ($y = 15.375x + 0.0009$, $R^2=0.998$); the results are given as the equivalent of mg catechin.

Determination of antioxidant activity using the ABTS method

Antioxidant activity of the samples was analysed using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) method (Chen *et al.,* 2018). The 7 mM ABTS solution containing 2.45 mM $K_2S_2O_8$ was prepared to determine antioxidant activity. This solution was held at room temperature in a dark setting for at least 12-16 hours to form $ABTS.^+$ radical solution. The radical solution was diluted with phosphate-buffered saline solution to give an absorbance value of 0.700 (±0.02) at 734 nm. Then, 1 ml of $ABTS.^+$ radical solution was taken, with 10 µl of sample extract added to and mixed gently with the radical solution. After 6 minutes, absorbance values were recorded. The inhibition ratio was found by calculating the percentage reduction of the $ABTS.^+$ solution of the initial absorbance value. Then, by changing the sample volume (10, 20, 30, 40 µl), the same procedures were repeated. The slope of the curve was plotted against the sample volumes corresponding to the inhibition ratio of the samples in different quantities. The results were expressed in terms of TEAC (Trolox equivalent antioxidant capacity) as µmol Trolox/ml, and was calculated by proportioning the slope of the graph of the linden tea samples to the slope of the standard curve prepared with Trolox ($y = 3.7932x + 4.6422$, $R^2=0.994$).

Hydroxymethylfurfural analysis

HMF analysis of flower honey and linden tea were carried out on high performance liquid chromatography (Agilent 1260 Infinity, Palo Alto, CA, USA). Samples were filtered through polyvinylidene fluoride with a pore size of 0.45 µm and transferred to amber-coloured 1.5 ml glass vials used in the HPLC sampling unit, ultimately being injected into HPLC. Methanol: water (10:90, v/v) mixture was used as the mobile phase. A diode array detector was used, 20 µl sample was injected at 285 nm at a flow rate of 1 ml/min, and measurements were taken at the end of a 25-minute

analysis. HMF peaks from chromatograms were calculated according to the standard curve (y = 124.09x – 1.1371, R^2=0.999) generated by injecting 1-5 µg/ml standard HMF solutions at 6 different concentrations (Chakir *et al.,* 2016).

Statistical analysis

All analytical determinations were repeated. The statistical analysis was performed using SPSS (version 22). The differences of mean values among samples were determined using one-way analysis of variance (ANOVA) followed by Bonferroni.

3. Results and discussion

Table 1 displays the total phenolic compounds, total flavonoid, total antioxidant activity, and HMF contents of the linden tea samples, which were prepared with different methods. Honey was also added at different times for each sample.

Total phenolic compounds of linden tea prepared by infusion and decoction were determined to be 0.16 and 0.24 mg GAE/ml of linden tea, respectively. The effect of different methods on the phenolic content is statistically significant [F(7,167.50)<0.001, *P*<0.05].

In one study, the total phenolic compounds of linden tea prepared by infusion were found to be 32.22 mg GAE/l, which is lower than the value determined in this study (Karakaya and El, 2006). In another study, 38.2 mg GAE/2 g dry linden was found, a value similar to the results of this experiment (Gil *et al.,* 2011). Total flavonoid content of linden tea prepared by infusion was 0.09, while total flavonoid content of the tea prepared by decoction was 0.12 mg CE/ml of linden tea. The effect of different methods on total flavonoid content is statistically significant [F(740.56)=0.001, *P*<0.05]. The total antioxidant activity values of linden tea prepared by infusion and decoction were found to be 1.07 and 1.30 µM Trolox/ml, respectively. The effect of different methods on total antioxidant activity is statistically insignificant [F(13.53)=0.067, *P*>0.05]. In a study in which total antioxidant activity was determined by the TEAC method, 84.7 µM Trolox/g linden flower was found. Calculation according to linden flower is close to the results (Kariotia *et al.*, 2014). Total antioxidant activity of linden tea prepared by different methods was found to be higher than that of teas prepared by decoction only. Total flavonoid and total catechin contents of linden tea prepared from various linden varieties prepared by infusion and decoction in a study were found to be higher in samples prepared using the decoction method (Kariotia *et al.,* 2014). Linden tea prepared by decoction could potentially increase the tea's medical effects (Zeybek and Haksel, 2010).

By adding honey to the linden tea prepared by infusion, the total phenolic compounds increased. The effect of the addition of honey to linden tea at different temperatures on total phenolic compounds is statistically significant [F(229.93)<0.001, *P*<0.05]. The addition of honey to linden tea at different temperatures has a statistically insignificant effect on total flavonoid content [F(0.092)=0.961, *P*>0.05]. The total flavonoid content of different varieties of flower honey in Turkey was examined. In two studies, values were found to be 0.65-8.10 mg quercetin/100 g honey (Can *et al.,* 2015) and 9.58-22.45 mg quercetin/100 g honey (Boussaid *et al.,* 2014). Since the amount of honey added is low, it is plausible that honey linden tea does not have a big effect on the total flavonoid value. By adding honey to linden tea prepared by this method, the total antioxidant activity

Table 1. Total phenolic, total flavonoid, total antioxidant and hydroxymethylfurfural (HMF) compound of linden tea samples.[1,2]

Method	Samples	Total phenolic compounds (mg GAE/ml)	Total flavonoid mg (CE/ml)	Total antioxidant (µM Trolox/ml)	HMF (µg/ml)
Infusion	linden flower tea	0.16±0.00^{*c}	0.09±0.00^{a}	1.07±0.04^{c}	nd
	linden flower tea instantly adding honey	0.17±0.00^{b}	0.08±0.00^{a}	1.08±0.06^{c}	nd
	linden flower tea after waiting 5 minutes + honey	0.19±0.00^{a}	0.08±0.00^{a}	1.12±0.09^{c}	nd
	linden flower tea after waiting 10 minutes + honey	0.19±0.00^{a}	0.08±0.00^{a}	1.73±0.08^{b}	nd
	linden flower tea after waiting 15 minutes + honey	0.18±0.00^{a}	0.08±0.00^{a}	2.17±0.13^{a}	nd
Decoction	linden flower tea	0.24±0.00^{a}	0.12±0.00^{a}	1.30±0.08^{b}	nd
	linden flower tea instantly adding honey	0.24±0.00^{a}	0.11±0.00^{b}	1.37±0.08^{b}	0.56±0.01^{a}
	linden flower tea after waiting 5 minutes + honey	0.24±0.00^{a}	0.11±0.00^{b}	1.46±0.04^{ab}	0.50±0.01^{b}
	linden flower tea after waiting 10 minutes + honey	0.24±0.00^{a}	0.11±0.00^{b}	1.80±0.13^{a}	0.50±0.00^{b}
	linden flower tea after waiting 15 minutes + honey	0.23±0.00^{b}	0.10±0.00^{b}	2.14±0.16^{a}	0.48±0.03^{b}

[1] Different letters in each column denote significant differences (*P*<0.05). Values are given as mean ± standard deviation.
[2] CE = catechin equivalents; GAE = gallic acid equivalents; nd = not detected.

values increased, and as the honey added temperature decreased, antioxidant activity increased. The effect of honey supplementation on total antioxidant activity at different temperatures was statistically significant. [F(61.45)=0.001, P<0.05]. In a study that added honey instantly to linden tea, the antioxidant activity of honey added tea also increased. In addition, linden tea with added pine honey showed a higher increase than added flower honey (Özdatlı *et al.,* 2014). A study was made by adding three different honey varieties (light amber, amber, and dark amber) to the lemon flavoured tea. Tea with only light amber honey added showed no change in flavonoid levels, and the amount of phenolic content increased a little. A large increase in total antioxidant capacity was observed when honey was added. The addition of different honey varieties was observed to affect the total antioxidant activity (dark amber > amber > light amber) (Pereira *et al.,* 2013). HMF was not detected in all samples with honey added at different temperatures and prepared by infusion.

The effect of the addition of honey at different temperatures on the total phenolic compounds of linden tea prepared by the decoction method is statistically significant [F(81.11)<0.001, P<0.05)]. However, the addition of honey to linden tea did not increase the total amount of phenolic content. The effect of the addition of honey at different temperatures on the total flavonoid content was found to be statistically insignificant for linden tea prepared by the same method [F(5.207)=0.072, P>0.05]. The effect of adding honey at different temperatures on total antioxidant activity was found to be statistically significant [F(21.12)=0.006, P<0.05]. The values increased when honey was added to the teas. The highest antioxidant value was obtained by adding honey at the lowest temperature after a waiting time of 15 minutes HMF was found in all samples prepared by decoction and when honey was added to them. The effect of adding honey at different temperatures on HMF formation was statistically significant [F(11.514)=0.019, P<0.05]. The highest HMF formation was observed in the linden tea with honey added at the highest temperature. In addition, the HMF value of honey was examined and found to be 4.99 µg/g. As mentioned in the Codex Alimentarius Honey standard, the amount of HMF in processed and/or blending honey should not be more than 40 mg/kg. Honey's HMF content is suitable according to the standard (FAO, 1981). In a 240 ml sample of linden tea, the amount of HMF in honey was theoretically calculated as 0.371 µg/ml. The results show that all the teas' HMF content obtained by decoction and supplemented with honey are above this value. Thus, the amount of HMF in teas is increased by the addition of honey, the decoction method and the water temperature. While the amount of HMF in fresh honey is very low, the amount of HMF increases due to heating duration, temperature, and storage (Yıldız *et al.,* 2010). It can be considered that the temperature of honey added to the decoction tea is high and the formation of HMF is observed due to the temperature while cooling.

4. Conclusions

In this study, it was observed that linden tea prepared differently by the infusion and decoction methods had higher total phenolic compounds, total flavonoid, and total antioxidant capacity than linden tea prepared by the decoction method. Also, the addition of flower honey at different temperatures did not cause an increase in the total flavonoid content of the samples prepared by both methods, but it resulted in an increase in the amount of phenolic content. The total antioxidant activity was increased in all tea samples supplemented with honey and the highest antioxidant activity was observed in the honey added at the lowest temperature. In addition, HMF formation was investigated in all linden teas. HMF was not detected without added honey and those prepared by infusion with added honey. However, HMF was detected in all linden tea with honey added that was prepared by decoction. The highest amount of HMF was detected in the linden tea with honey added at the highest temperature. As a result, at least 10 minutes should pass after preparation before adding honey to linden tea. Thus, total antioxidant activity is increased while HMF formation is reduced. In the future, it is suggested that studies should be conducted with an increased variety of different honey and plant types to understand the effect of these mechanisms.

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