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Phenolic content and antioxidant activity of linden syrup prepared from dried flowers by hot and cold brewing

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Linden flower syrup has been used as a supportive treatment for colds in folk medicine. In this study, syrup was prepared by mixing sucrose and herbal infusion made by hot (HB) and cold (CB) brewing from air-dried (AD) and freeze-dried (FD) linden flowers. The syrup was preserved by heating at 75 °C for 25 min followed by storage at 5°C for 21 d. The colour, pH, phenolic content, and antioxidant activity were determined. Hot brewing produced syrups with a significantly higher amount of phenolics, flavonoids, and antioxidant activity than cold brewing. The contents of catechin (39.59 mg/L), epicatechin (159.07 mg/L), chlorogenic acid (44.66 mg/L) and tiliroside (1.92 mg/L) contents were higher for HB-FD than those of the HB-AD samples. Regarding cold brewing, it was found to effectively extract catechin, epicatechin, and chlorogenic acid from air-dried flower samples.

Keywords: *Tiliae flos*; HPLC analysis; Sugar solution

Introduction

Linden flower, commonly known as a lime flower or under the official classification name as *Tiliae floss*, is a plant material with a yellowish-green colour and an aromatic scent. The main constituents of the linden flower are polysaccharides, flavonoids (quercetin glycosides, kaempferol glycosides, tiliroside), phenolic acids, essential oils, phytosterols, organic acids, tannins such as procyanidin dimers (B−2), mucilage, minerals, niacin, and vitamin C [1]. Studies have been carried to address the effect of linden and its constituent upon

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human health. Linden is an excellent remedy for stress and panic attacks, and its constituents are proven to have anti-inflammatory activity [2]. Also, this substance relieves tension and sinus headaches, thus helping calm the mind and allowing easy sleeping [3,4]. As recently observed, *Tilia* honey was responsible for suppressing influenza A virus by regulating the immune response of macrophages [5]. Freeze-dried products are believed to have the same characteristics as fresh ones. As such, the preservation and retention of attributes like the shape, appearance, taste, nutrients, porosity, colour, flavour, texture, and biological activity of fresh samples makes this technique one of the most fascinating and applicable to dry food materials [6]. Freeze-dried plant materials usually show higher content of phenolic constituents and antioxidant properties compared to the oven- or sun-dried counterparts [7], but the opposite findings are also documented [8,9]. When an extract of this dried plant material is prepared, the drying method can influence the evolution of phenolic substances during storage [10,11]. Therefore, it is useful to know about how the drying affects antioxidant properties of the extract during storage. A sugar solution (syrup) is a conventional carrier for various pharmaceutical sub-stances that can be particularly effective for children between the ages of 4 and 10 [12]. Moreover, preparation of syrup is the only way of how to preserve the active ingredients of the herb extract in the domestic environment. This is due to the decrease in water activity, ensuring microbial safety during storage.

This study aims to compare some physical (pH, colour) and chemical properties (antioxidant capacity, phenolic content) of linden syrup prepared by hot and cold brewing from air-dried and freeze-dried flowers.

Materials and methods

Chemical reagents

Crystal sucrose (Cukrovar Vrbátky a.s., Czech Republic) was purchased in the local store. The following chemicals were of analytical grade (purity ≥90 %, Sigma Aldrich, Steinheim am Albuch, Germany): 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), Folin-Ciocalteau reagent (FC), 6-hydroxy-2,5,7,8-tetramthylchroman-2-carboxylic acid (Trolox), gallic acid, quercetin, (+)-catechin, (−)-epicatechin, chlorogenic acid, rutin hydrate, tiliroside, ferric chloride and aluminium chloride. Formic acid (≥95 %) and methanol Chromasolv® were used for HPLC analysis.

Sample collecting and drying

Flowers with bracts were manually harvested from a linden tree (*Tilia ptalophyllos*) on 30th of July 2020 (Jilemnice, Czech Republic, 465 m above sea level). The flowers were dried at 35 °C for 14 h using food dehydrator Gallet DES 120 (AD) or at −110°C for 24 h (FD) using freeze dryer L4-110 (Gregor Instruments s. r. o., Říčany, Czech Republic). Drying time periods were optimized to ensure a low final moisture level (<10 %). Both dried samples were manually cut and stored in glass jars at −20 °C until further analysis.

Preparation of linden syrup

Herbal infusions were prepared by hot (HB) and cold (CB) brewing, i.e. 10.0 g of dried sample was mixed with 250 mL of hot (90 °C for 5 min) and cold (25 °C) distilled water, respectively. The mixtures were kept in the dark at laboratory temperature (20.8–23.4 °C) for 24 h, then passed through a sieve to remove solids. Linden flower infusion was carefully heated with the successive addition of sucrose to a final ratio of 200 g per 250 mL followed by boiling linden syrup for 5 min. Four types of linden syrup were referred to as HB-AD, HB-FD, CB-AD, and CB-FD; i.e., for example, hot (AD-HB) or cold brewed air-dried (AD-CB) samples. The syrup was cooled in an ice bath, and aliquots stored at −20 °C for chemical analysis. This syrup preparation procedure is common in traditional medicine. The colour, pH, and water activity were immediately measured after cooling the samples.

Determination of physical parameters of linden syrup

The pH of the samples was measured using Polylite Lab glass electrode (type HF Glass, Hemilton, Reno, NV, USA) using a conventional pH-meter CG 842 (Shott Glas, Mainz, Germany). An Aqualab TDL meter (Pullman, WA, USA) was used for the measurement of water activity at 25 °C. The transmitted colour of linden syrup was determined using UltraScan VIS spectrophotometer (HunterLab, Reston, VA, USA) with d/8° geometry, D65 light source, and 10 mm of path length. The colour was expressed using the CIELAB colour system, where L^* represents a value from 0 (black) to 100 (white), a^* value indicates red (+) and green (−) colour components. The yellow and blue components are described by a positive and negative value of b^*, respectively. All the measurements were done in triplicate.

Antioxidant properties of linden syrup

The reaction of 5.0 mL of DPPH methanol solution (2.5%, w/v) and 500 μL of the diluted sample took place in a dark chamber for 20 min. The change in absorbance was monitored at a wavelength of 517 nm using an UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). The so-called ferric reducing antioxidant capacity (FRAP) was performed to test the ability of linden syrup to reduce ferric ions. Briefly, a reagent solution was prepared by mixing 20 mM $FeCl_3$, 10 mM TPTZ in 40 mM HCl, and acetic buffer solution (3.6 pH) in a ratio of 1:1:10 [13]. A sample (300 μL) was added to 3.0 mL of reagent solution and allowed to stand for 50 min in a dark cabinet, followed by observing the absorbance at 593 nm. The DPPH and FRAP assay results were expressed as Trolox equivalent capacity (mg/L). An equivalent of sucrose solution (200 g per 250 mL) was used in both assays for control.

Phenolic content in linden syrup

Total phenolic content (TPC) was determined using Folin-Ciocalteau method as described in literature [13]. One millilitre of samples was mixed with 0.5 mL of FC reagent and 6.0 mL of distilled water. The mixture was left alone for 5 min, then 1.0 mL of 5% (w/w) of sodium carbonate was added, followed by incubation for 90 min in a dark place. The increase in absorbance was observed at 765 nm. The results were expressed as gallic acid equivalent (mg GAE/L). The mixture with sucrose solution served as a control. Total flavonoid content (TFC) was estimated using aluminium chloride assay with minor modification [14]: A mixture consisting of a diluted sample (2.0 mL), 2.0% AlCl3 (1.0 mL), 1.0 M HCl (1.0 mL), 1.0 M acetic acid (1.0 mL), and 1.0 mL of distilled water was allowed to stand in quiet for 30 min, then absorbance was checked at 425 nm. The results were expressed as the quercetin equivalent (mg QUE/L).

HPLC analysis of phenolic compounds

Analyses were performed using a liquid chromatographic system Nexera X2 (Shimadzu, Kyoto, Japan) equipped with a degasser DGU-20A5R, binary gradient pump LC-30AD, autosampler SIL-30AC, column oven CTO-20AC and photo diode array (PDA) detector SPD-M30A. Analytes were separated on 100 × 2.1 mm Ascentis® C18 column filled with 3-μm fully porous particles (Supelco, Bellefonte, PA, USA). Mobile phase from deionized water acidified with formic acid (pH ~ 3.2, solvent A) and pure MeOH (solvent B) was selected. Separation was carried out at 40 °C, with flow rate of 0.5 mL min^{-1} and injection volume of 10 μL. The following conditions of gradient elution were then applied: 0 min 5 % B,

10 min 12 % B, 35 min 55 % B and 40 min 90 % B. Signal was detected at 260 nm (rutin), 280 nm ((+)-catechin and (−)-epicatechin), 315 nm (tiliroside) and 325 nm (chlorogenic acid) depending on absorption maximum of each compound. Before measurement, all samples of linden blossom syrups were three-times diluted with an initial composition of mobile phase and filtrated through 0.22 μm PTFE syringe filter. For the preparation of calibration standards, solid compounds (tiliroside, chlorogenic acid, rutin, (−)-epicatechin and (+)-catechin) were dissolved in pure MeOH.

Statistical analysis

The results were expressed as the mean ± standard deviation. Two-way analysis of variance (ANOVA) was applied to test the effect of drying method (air-dried vs. freeze-dried, factor A) and brewing (cold vs. hot, factor B) on functional characteristics of linden syrup. A post-hoc Duncan multiply pairwise comparison test was used to assess the difference between the means. Principal component analysis (PCA) was applied to determine the overall effect of variables on properties of linden flower syrup samples. All the statistical procedures were done at probability level $p = 0.05$ (Statistica v. 14.0, Tibco, Palo Alto, CA, USA).

Results and discussion

The effect of drying and brewing on pH and colour of linden flower syrup

In this study, linden infusions were prepared by hot and cold brewing of air-dried and freeze-dried linden flowers; both in water. Afterwards, the extracts were boiled with sucrose to prepare linden syrups. Some physicochemical properties are listed in Table 1. The pH values ranged from 5.16 ± 0.02 to 5.61 ± 0.03 and being significantly lower for syrups prepared from FD ($p < 0.001$) than for AD samples. Cold brewing technique resulted in a considerably lower pH ($p < 0.01$) of linden syrup than that of the hot brewing. It is known that powder prepared by different drying methods has a different effect on the pH of the final product due to variations in the content of organic acids [15]. Conventionally dried reconstituted powder (45 °C, 48 h) of kedondog powder exhibited a higher pH than their freeze-dried counterparts [16], which was consistent with our study. The decomposition of organic acids can also explain the higher pH of linden syrup prepared by hot brewing in our research.

Table 1 The colour and pH of linden flower syrup prepared from air-dried (AD) and freeze-dried (FD) flowers by hot (HB) and cold (CB) brewing

		pH	L^*	a^*	b^*
HB	AD	5.61 ± 0.03^{a}	93.41 ± 0.50^{a}	-2.18 ± 0.06^{b}	24.85 ± 0.07^{b}
	FD	5.27 ± 0.05^{c}	93.73 ± 0.06^{a}	-1.69 ± 0.05^{a}	18.06 ± 0.39^{d}
CB	AD	5.36 ± 0.06^{b}	93.54 ± 0.17^{a}	-1.84 ± 0.03^{a}	21.82 ± 0.37^{c}
	FD	5.16 ± 0.02^{d}	88.73 ± 1.41^{b}	-2.55 ± 0.47^{b}	39.71 ± 2.57^{a}

Different letters in superscript indicate significant differences in column using Duncan test ($p < 0.05$)

The colour of linden syrup was influenced by both brewing and drying techniques. The same values of L^* ($p < 0.05$) were observed for linden syrup samples prepared by hot brewing of AD and FD linden flowers (93.41 ± 0.50 and 93.73 ± 0.06, respectively), whereas cold brewing of AD linden flowers gave rise to a syrup with significantly higher lightness ($L^* = 93.54 \pm 0.17$) than that of freeze-dried samples ($L^* = 88.73 \pm 1.41$; $p < 0.001$). Regarding hot brewing, the FD syrup showed significantly higher value of a^* (-1.69 ± 0.05; $p < 0.05$) and lower value of b^* (18.06 ± 0.39; $p < 0.001$) values when compared to the AD syrups ($a^* = -2.18 \pm 0.06$, $b^* = 18.06$). An opposite effect was found for linden syrup made from CM extract, i.e. significantly lower values of a^* (-2.55 ± 0.47; $p < 0.01$) and higher b^* (39.71 ± 2.57; $p < 0.001$) were determined for the syrup manufactured from freeze-dried than for air-dried flowers.

Drying and brewing effect on antioxidant activity and phenolic content of linden flower syrup

The total phenolic (TPC) and flavonoid (TFC) contents of linden flower syrup ranged from 309.13 to 909.37 mg GAE/L and from 19.9 to 31.8 mg QUE/L, respectively (Table 2). As reported in a recent work by Preti and Tarola [17], linden honey samples contained 318 mg GAE/kg, corresponding to the TPC of syrup samples prepared from cold brew in our study. TFC values ranged from 14.8 to 25.6 mg QUE/kg for six samples of linden honey from Romania [18], which was similar to our findings. Furthermore, the TPC values differed significantly between the samples in the order HB-AD > HB-FD > CB-AD > CB-FD, whereas the total content of flavonoids remained at the same level. Epicatechin (47.30–159.02 mg/L) and chlorogenic acid (23.17–40.66 mg/L) were the most abundant phenolic constituents in our linden syrup samples. This is in accordance with the study by Ziaja et al. [19], who found (−)-epicatechin as the dominant phenolic compound among 74 *Tilia* flower samples. As can be seen, all the phenolic contents and antioxidant properties were significantly higher for HB ($p < 0.05$) than for CB linden syrup samples regardless of drying method. More

phenolic substances and higher antioxidant activity were obtained by hot extraction (98 °C for 16 min) of edible roselle flowers compared to cold maceration for 24 h [20]. In contrast, cold water was more efficient for the extraction of phenolic substances from roselle than hot water [9]. Furthermore, more phenolics were obtained from room-dried (27 °C, 4 days) and sun-dried (35 °C, 1 day) samples than those from freeze-dried samples in their study.

Table 2 Phenolic content and antioxidant properties of linden flower syrup prepared from air-dried and freeze-dried flowers by hot and cold brewing

	Hot brewing		Cold brewing	
	Air-dried	Freeze-dried	Air-dried	Freeze-dried
TPC	909.4 ± 106.2^{a}	867.7 ± 62.9^{b}	442.6 ± 18.5^{c}	309.1 ± 16.3^{d}
TFC	27.5 ± 3.1^{a}	31.8 ± 0.6^{a}	22.1 ± 0.4^{b}	19.9 ± 0.5^{b}
Phenolics	mg/L			
cat	29.87 ± 0.91^{b}	39.59 ± 0.84^{a}	23.97 ± 0.36^{c}	20.74 ± 0.17^{d}
epi	85.72 ± 1.58^{b}	159.02 ± 7.41^{a}	58.41 ± 2.37^{c}	47.30 ± 2.90^{d}
chla	36.84 ± 0.42^{b}	40.66 ± 0.18^{a}	30.31 ± 0.07^{c}	23.17 ± 0.50^{d}
rutin	25.32 ± 0.90^{a}	29.13 ± 3.11^{a}	12.38 ± 0.27^{b}	14.80 ± 1.43^{b}
tiliroside	1.52 ± 0.03^{b}	1.92 ± 0.11^{a}	0.30 ± 0.01^{d}	0.73 ± 0.04^{c}
DPPH	2.37 ± 0.34^{a}	2.29 ± 0.27^{a}	1.26 ± 0.04^{b}	0.622 ± 0.06^{c}
FRAP	1.68 ± 0.08^{a}	1.62 ± 0.02^{a}	0.93 ± 0.05^{b}	0.48 ± 0.03^{c}

TPC, total phenolic content (mg GAE/L); TFC, total flavonoid content (mg QUE/L); GAE, gallic acid equivalent; QUE, quercetin equivalent; cat, (+)-catechin; epi, (−)-epicatechin; chla, chlorogenic acid; DPPH and FRAP expressed as g Trolox/L; different letters in superscript indicate significant differences in row using Duncan test ($p < 0.05$)

In our study, the effect of the drying method applied to prepare linden flowers did not show an apparent trend ($p < 0.05$). In the case of hot brewing, significantly higher contents of catechin, epicatechin, chlorogenic acid, rutin, and tiliroside were observed for HB-FD syrup. On the contrary, total phenolic content, total flavonoid content, DPPH, and FRAP were similar for both HB-AD and HB-FD linden syrup samples. This can be explained by the release of other phenolic compounds during air-drying process, which then may increase the antioxidant effect of HB-AD linden syrup. For example, caffeic acid, astragalin, quercitrin, hyperoside, and two other polar compounds with antiradical scavenging activity were determined in ethanolic extracts of air-dried linden flowers [21]. Interestingly, differences in phenolic content were of varying magnitude, that is, the epicatechin content was lower by 85% for air-dried samples, and catechin, chlorogenic acid, rutin, and tiliroside showed only 10–32% reduction.

The great loss of epicatechin during the heat treatment has been well-documented for banana flour [22] or hawthorn slices [23]. On the other hand, cold water was more favourable to the release of epicatechin ($p < 0.01$), catechin ($p < 0.001$) and chlorogenic acid ($p < 0.001$) from the AD linden flower syrups after 24 h of maceration compared to the freeze-dried sample. It also corresponded to significantly higher TPC value (442.62 mg GAE/L; $p < 0.05$) and antioxidant properties in terms of DPPH (1260.0 mg TEA/L; $p < 0.01$) and FRAP (926.2 mg TEA/L; $p < 0.001$) assays.

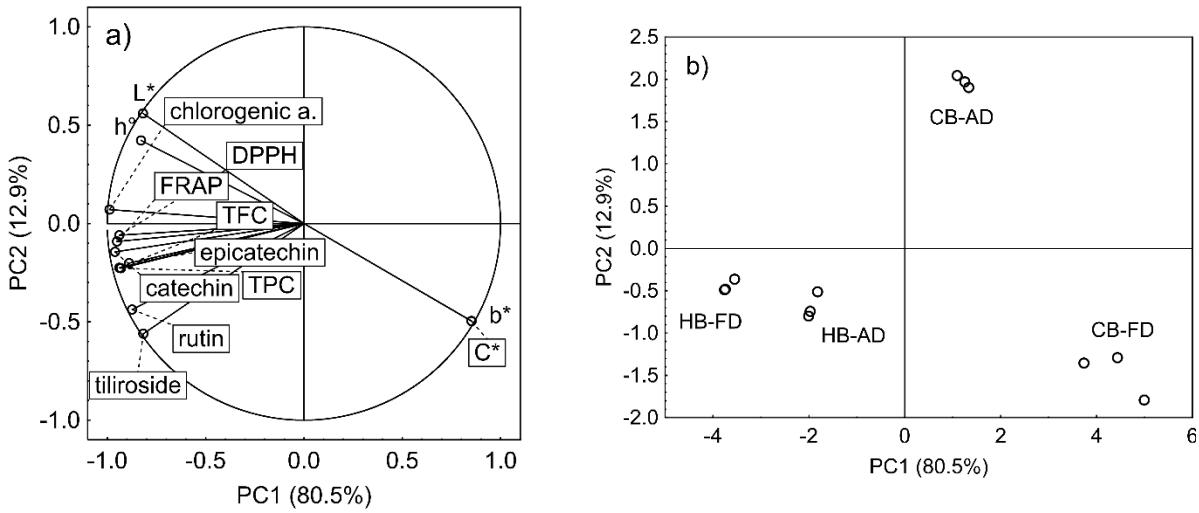

Fig. 1 Linden flower syrup variables as affected by air-drying (AD), freeze-drying (FD), and hot (HB) and cold brewing (CB) in corresponding (a) principal loading and (b) scatterplot

Principal component analysis (PCA) was applied to elucidate the mutual association between variables for linden flower syrup samples. The scree plot identified two components above eigenvalue one, sufficient to describe 93.4% of the total variance of the data. Two variables (pH and a^*) were excluded from the principal component analysis due to the short lengths of vectors, indicating the redundancy of these parameters. The loadings of the main components (Figure 1a) showed that the first component (PC1) is predominantly given by antioxidant properties, particularly the content of TFC, TPC, FRAP, DPPH, and of catechin (vectors are parallel to the x-axis). The vectors of the other variables formed acute angles with the x-axis, indicating a strong positive correlation with PC1 explaining 80.5 % of the data variance. The second principal component (PC2) is described by the colour and partially by the content of tiliroside plus rutin that cover 12.9 % of the variance in the data. As can be seen in the corresponding scatterplots (Figure 1b), HB syrup samples were located along the negative PC1 axis, confirming their distinctive antioxidant properties and phenolic contents compared to the CM syrup samples. In addition, HB-FD and HB-AD samples also formed distinct groups along the PC1. This confirms that

syrup prepared by hot brewing of FD linden flower has higher antioxidant properties. CB-AD and CB-FD samples were separated along PC2, having negative and positive values, respectively. This can be attributed to a different colour, predominantly based on b^* (blue-yellow) value. In general, the parameters L^* and a^* were positively associated with antioxidant properties, while negative effects were ascertained for values of b^*. The correlation analysis between antioxidant properties and colour parameters is presented in Table 3.

Table 3 Correlation coefficient between colour, and phenolic and antioxidant contents

	DPPH	TPC	TFC	FRAP
L^*	0.764^{++}	0.660^{+}	0.627^{+}	0.753^{++}
a^*	0.497	0.416	0.549	0.509
b^*	-0.745^{++}	-0.654^{+}	-0.709^{+}	-0.742^{++}

Statistical significance of correlation coefficient is denoted as $^{+}$ ($p < 0.05$) and $^{++}$ ($p < 0.01$)

Conclusions

In this study, different drying and brewing methods have been used for the preparation of linden flower syrup. Freeze-dried flower and cold brewing led to a small but still significant decrease in pH. Hot brewing gave a syrup with significantly higher phenolic and flavonoid contents and antioxidant activity compared to cold brewing, regardless of the drying method. Epicatechin, chlorogenic acid, and catechin were the most abundant phenolic constituents in all samples of syrup. However, the amounts of catechin, epicatechin, chlorogenic acid, and tiliroside were higher for syrups prepared from freeze-dried than those of air-dried flowers when hot-brewed. On the contrary, cold brewing effectively extracted catechin, epicatechin, and chlorogenic acid from air-dried flower samples. When using principal component analysis, phenolic and antioxidant content, as well as colour have clearly differentiated among all the samples of linden flower syrup.

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