

Variation on the terpene profile of *Ocimum basilicum* leaf tea caused by leaves storage conditions and preparation methods

Variação no perfil de terpenos do chá de folhas de *Ocimum basilicum* causada pelas condições de armazenamento das folhas e métodos de preparo

Variación en el perfil de terpenos del té de hojas de *Ocimum basilicum* causada por las condiciones de almacenamiento de las hojas y los métodos de preparación

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Abstract

The use of teas made by medicinal plants is a consolidated practice in Brazil. The chemical profile of teas can be affected by the storage and handling conditions of the plant material. These conditions can cause volatilization, enzymatic degradation, hydrolysis and oxidation of the secondary metabolites, which can affect the biological properties of the teas. This paper describes how the chemical profile of basil extracts, prepared by aqueous infusion, is affected by variations on the contact time between plant material and boiling water (5, 10 20 and 30 minutes), and the use of whole and fragmented fresh leaves, that were stored according to the methods commonly used by the population: cooling and freezing (7 days). The chemical profile of the extracts was evaluated by GC-MS. The tea in which the highest number of metabolites was extracted, 15 in total, was obtained using fresh fragmented leaves with 20 minutes of infusion. In the teas prepared by using whole fresh leaves (infusion by 5 to 30 minutes) and frozen leaves (infusion by 30 minutes), 11 metabolites were observed. The teas prepared by using cooled leaves (infusion by 5 to 30 min) and dried leaves (infusion by 30 min) presented 5 metabolites each. In the tea prepared by using dried leaves, with infusion time of 5 minutes, only 2 metabolites were observed. The results indicate that the presence of pharmacological bioactive metabolites in homemade basil's tea may vary according to the form of storage of the leaves and preparation methods of the tea.

Keywords: GC-MS; Chemical profile; Basil (*Ocimum basilicum*); Medicinal plants.

Resumo

No Brasil o emprego de plantas medicinais é uma prática consolidada. O perfil químico dos chás pode ser afetado pelas condições de armazenamento e manipulação do material vegetal, que podem favorecer processos de volatilização, degradação enzimática, hidrólise e oxidação dos metabólitos secundários, afetando suas propriedades biológicas. Neste trabalho foi investigado como o perfil químico dos extratos de manjericão, preparados por infusão aquosa, é afetado por variações no tempo de contato entre material vegetal e água fervente (5, 10 20 e 30 minutos), e emprego de folhas frescas inteiras e fragmentadas e folhas que foram armazenadas de acordo com os métodos

comumente empregadas pela população: secagem, resfriamento e congelamento (por 7 dias). O perfil dos extratos foi avaliado por CG-MS. O chá no qual ocorreu a extração de maior número de metabólitos, 15 no total, foi obtido empregando folhas frescas fragmentadas com 20 minutos em infusão. Nos chás que empregaram folhas frescas inteiras (infusão por 5 a 30 minutos) e folhas congeladas (infusão por 30 min.) foram observados 11 metabólitos. Nos chás obtidos a partir de folhas resfriadas (infusão por 5 a 30 min.) e das submetidas a secagem (infusão por 30 min.), foram observados 5 metabólitos. Já para folhas secas, tempo de infusão de 5 minutos, apenas 2 metabólitos foram observados. Tais resultados indicam que nas preparações caseiras de chás de manjericão, a presença de metabólitos com efeitos farmacológicos pode variar conforme a forma de armazenamento das folhas e preparo dos chás.

Palavras-chave: GC-MS; Perfil químico; Manjericão (*Ocimum basilicum*); Plantas medicinais.

Resumen

En Brasil, el uso de plantas medicinales es una práctica consolidada. El perfil químico de los té pude verse afectado por las condiciones de almacenamiento y manipulación del material vegetal, que pueden favorecer procesos de volatilización, degradación enzimática, hidrólisis y oxidación de los metabolitos secundarios, afectando sus propiedades biológicas. En este trabajo se investigó cómo el perfil químico de los extractos de albahaca, preparados por infusión acuosa, se ve afectado por variaciones en el tiempo de contacto entre material vegetal y agua hirviendo (5, 10, 20 y 30 minutos), y por el uso de hojas frescas enteras y fragmentadas, y de hojas que se almacenaron según los métodos comúnmente empleados por la población: secado, enfriamiento y congelación (durante 7 días). El perfil de los extractos se evaluó mediante GC-MS. El té que se extrajo mayor número de metabolitos, 15 en total, obtuvo utilizando hojas frescas fragmentadas y en infusión durante 20 minutos. Los té que emplearon hojas frescas enteras (infusión de 5 a 30 minutos) y hojas congeladas (infusión de 30 minutos), observaron 11 metabolitos. Las infusiones obtenidas a partir de hojas enfriadas (infusión de 5 a 30 minutos) y a secado (infusión de 30 minutos), observaron 5 metabolitos. Para hojas secas, con un tiempo de infusión de 5 minutos, solo se observaron 2 metabolitos. Estos resultados indican que en las preparaciones caseras de té de albahaca, la presencia de metabolitos con efectos farmacológicos puede variar según la forma de almacenamiento de las hojas y la preparación de los té.

Palabras clave: GC-MS; perfil químico; Albahaca (*Ocimum basilicum*); Plantas medicinales.

1. Introduction

To treat diseases and their symptoms, medicinal plants are used. Their pharmacological action is due to the presence of bioactive secondary metabolites: the phytochemicals (Sarri et al., 2022). In folk medicine, plants are used in their natural form or in preparations, amongst which teas are the most common (Nedopetalski & Krupek, 2020). Teas are prepared by the infusion process, in which the plant material is immersed in boiling water for a specified time to promote the extraction of phytochemicals (Gibertoni et al., 2020). The pharmacological action of a tea is related to its chemical profile, which can be affected by both the conditions of the plant material and the preparation methods (Goularte, Santos and Ziech, 2021). Between the time of harvest and the preparation of the phytochemical, losses of metabolites can be promoted by volatilization or degradation. The chemical degradation reactions can result from the interaction of the metabolites with the plant enzymes (present in the material itself), with the water or with oxygen. The exposure to light, air, humidity, and heat are crucial factors that should be observed during the plant material storage (Simões et al. 2017).

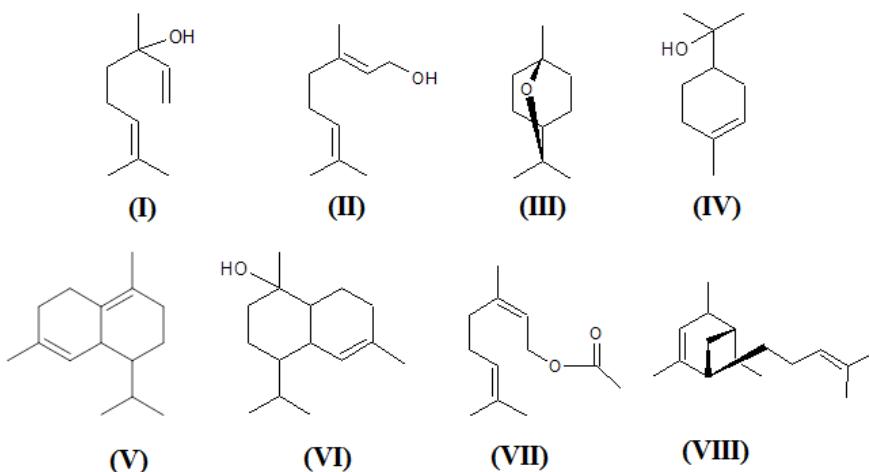
The boiling water immersion of the plant during the infusion process promotes the intumescence of the plant cells, improving the extraction of metabolites. However, the use of heat can be ambiguous since it can both favor the volatilization of low vapor pressure compounds and catalyze the degradation of thermolabile metabolites. Oliveira (2014) indicates that long time periods and high infusion temperatures can promote the oxidation of phenolic compounds, while short time intervals may not be sufficient for complete solubilization of the compounds of interest. Then, high temperatures can increase extraction efficiency, since heat makes cell walls permeable, increasing the solubility and diffusion of the compounds to be extracted. Thus, it is possible to assume that homemade teas may present different profiles of pharmacological bioactive metabolites according to the manner in which they are produced and the plant material is stored.

The basil (*Ocimum basilicum*) is a perennial plant, widely disseminated in Brazil, but originated in India. The popular knowledge used leaves and flowers of the basil to obtain teas for its tonic and digestive properties, in addition to helping in the treatment of respiratory and rheumatic problems. The same can also be used in popular cuisine in the form of a condiment

(Maciel et al., 2022). This species is also recognized as a medicinal plant, being used in the treatment of diseases such as hypercholesterolemia and hyperglycemia (Machado et al. 2011). Besides the pharmacological properties, there are other biological properties, such as insecticidal and pesticide (Umerie, Anaso and Anyasoro 1998), antimicrobial in grains and in beefs (Montes-Belmont and Carvajal 1998; Aquino et al. 2010).

Phytochemical studies indicate that *Ocimum basilicum* presents several terpenes, among which the most frequent are five monoterpenes: linalool (I), geraniol (II), 1,8-cineol (III), α -terpineol (IV), δ -cadinene (V) and three sesquiterpenes: α -cadinol (VI), geranyl acetate (VII) and α -trans-bergamotene (VIII) (Venancio 2006). Their structures can be seen in Figure 1.

Figure 1 - Structures of the main terpenes that compose the basil extracts.



Source: Own authorship. Software: ChemSketch (1994).

The literature reports several biological activities for some of those substances in Figure 1: linalool has analgesic and anti-inflammatory properties (Peana et al. 2002). Geraniol is repellent (Brito et al. 2015) and antitumoral (Carnesecchi et al. 2001). 1,8-cineole is decongestant and antitussive (Santos and Rao 2000). Terpineol exhibits antimicrobial effects on periodontopathic and cariogenic bacteria (Park et al 2012). δ -cadinene exhibits anti-inflammatory and sedative actions (Mohali, Padillha-Baretic and Rojas-Fermín 2013), antibacterial activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Burkholderia cepacia* and *Hafnia alvei*, and antifungal activity against *Aspergillus fumigatus* (González et al 2012). α -cadinol exhibits antifungal properties against *Laetiporus sulphureus* and *Coriolus versicolor* (Chang, Wang, and Kuo 2003) and antibacterial properties against *Staphylococcus aureus* and *Bacillus cereus* (Murari et al. 2008).

With the aim to verify if the presence of pharmacological bioactive metabolites in homemade basil teas could be varied accordingly to the way that the leaves are stored and the teas are prepared, the present study was developed. In this case, preparation of the extracts was performed by a similar way to the homemade recipes. Regarding storage, the plant was used fresh (fragmented and whole leaves), refrigerated (for 7 days), frozen (for 7 days), and dried (at 60° C until constant mass). Regarding the preparation conditions, the contact time between the plant material and boiling water varied from 5 to 30 minutes.

2. Material and Methodology

Material

Reagents

Ethyl Acetate (Brand: Vetec); Ethyl Alcohol (Brand: Vetec); Calcium Carbonate (Brand: Vetec); Sodium Chloride (Brand: Vetec) and Dichloromethane (Brand: Synth).

Equipments

Gas chromatograph coupled to a mass spectrometer - Brand: Agilent Technologies/Model: 7890A, coupled to a mass selective detector (Agilent Technologies 5975C inert MSD Triple-Axis Detector), with capillary column type DB-5MS (30 m x 0.25 mm x 0.25 µm); Digital balance - Brand: Bel Engineering; Vortex Mixer - Brand: Kasvi basic K45-2810; Centrifuge - Brand: Centrifuge 5410; Automatic pipette 100-1000 µL - Brand: Expendorf Research Plus.

Methodology

Acquisition of the plant material

Basil leaves were harvested in April of 2018, at 7:45 am in the city of Belo Horizonte (Minas Gerais, Brazil), according to the coordinates - 19°51'41.4 "S 43°59'09.5 "W.

Treatment and Storage of the plant material

The basil leaves, after being harvested, were separated into 5 portions of 10 g each. Two of these portions were sealed in transparent plastic bags, one of these was kept in the refrigerator for 7 days (about 8°C) and the other one was kept in the freezer for 7 days (about -17°C). Two other portions were used on the same day of collection to prepare the extracts, one of which was manually fragmented to about 1 cm. The last portion was dried in a kiln until it reaching a constant mass.

Extraction by infusion

To prepare each extract by infusion, distilled water was boiled until the boiling temperature and 5 mL was poured into 10 mL erlenmeyers. To each of the erlenmeyers 1.0 g of the following samples were added: whole fresh leaves, fragmented fresh leaves, leaves cooled for 7 days, leaves frozen for 7 days, and 0.3 g of dried leaves. The erlenmeyers were capped and aliquots were removed from each one after 5, 10, 20 and 30 minutes of contact between plant material and solvent. The entire process was performed in duplicate.

Dispersive Liquid-Liquid Microextraction

Dispersive liquid-liquid micro extractions were performed to the aqueous extracts of basil as described in the methodology presented in Caldas et al. (2011). The aqueous extracts were homogenized, and an automatic pipette was used to transfer 700 µL of the extract to the eppendorf. Then, 100 µL of ethanol as dispersing solvent and 250 µL of dichloromethane as extracting solvent, in addition to 1 mg of sodium chloride were also added. The mixture was taken to vortex for 1 minute for homogenization and finally to centrifugation for 2 minutes for phase separation. Subsequently, with a micro syringe, the organic phase was transferred to the micro vial for chromatographic analysis (Martins et al. 2012).

Chromatographic analysis

The chromatographic analysis was performed under the following conditions: fused silica capillary column (HP-MS), maintaining helium flow as a carrier gas, heating at a programmed temperature of 60°C for 2 minutes up to 110°C at

3°C min⁻¹, then up to 180°C at 12°C min⁻¹, maintained for 7 minutes, finally up to 200°C at 20°C min⁻¹ and maintained for 2 minutes.

Identification of the substances

To ensure the identification of the substance corresponding to each peak that was present at the chromatogram, the retention indices were calculated by the Kovats method and the mass spectra for each peak observed in the chromatogram was compared with those available in the instrument's database. The Kovats index values described in the literature and experimentally obtained were compared.

3. Results and Discussion

The list of all metabolites identified at the infusions and their Kovats index are shown in Table 1 and the chemical profile of each infusion is shown in Table 2.

Table 1 - Retention time and Kovats index for the metabolites identified in the basil infusion samples.

Metabolites	R.T. (min.)	TK	EK
β-pinene	6,8	943	949
limonene	8,3	1014	1015
eucalyptol	8,3	1023	1015
γ-terpinene	9,3	1059	1057
linalool	10,6	1101	1110
camphor	12,1	1160	1160
α-terpienol	13,5	1192	1205
eugenol	17,8	1363	1371
α-copaene	18,1	1397	1413
β-elemene	18,4	1405	1411
β-caryophyllene	18,8	1424	1423
α-humulene	19,3	1456	1466
germancrene B	19,7	1560	1565
δ-cadinene	20,2	1514	1520
germancrene-D-4-ol	21,2	1560	1552
α-cadinol	21,9	1627	1622

R.T. –Retention time; T.K. -Theoretical Kovats; E.K. -Experimental Kovats. Source: Own authorship.

Table 2 - Metabolites present in the various extracts prepared by infusion.

Metabolites	% area under. the peak													
	Leaf condition													
	Fragmented fresh				Whole fresh				Cooled (7 days)		Frozen (7 days)		Dried	
	Contact time of the leaves with boiling water (min.)													
	5	10	20	30	5	10	20	30	5	30	5	30	5	30
β-pinene	-	8,2	5,4	7,5	6,7	7,6	7,6	9,6	6,8	7,5	7,8	9,5		
	-	6,0	-	-	7,4	9,3	7,8	6,3	8,3	7,7	7,8	8,1		
limonene	-	63,6	54,9	-	65,0	68,3	64,5	73,6	72,4	71,7	71,6	75,1	69,7	67,6
	-	63,0	-	-	73,4	72,4	70,6	66,7	76,6	77,1	76,5	73,4	78,1	65,4
eucalyptol	22,3	-	-	66,1	-	-	-	-	-	-	-	-	-	
	24,4	-	26,8	3,6	-	-	-	-	-	-	-	-	-	
γ-terpinene	-	4,2	3,0	3,8	4,4	4,6	3,8	4,4	4,4	4,3	4,3	4,6	4,7	5,4
	-	4,1	-	-	4,7	4,6	4,6	4,4	4,6	4,5	4,8	4,3	6,7	5,1
linalool	11,6	5,1	6,3	5,6	-	-	-	-	6,3	4,6	2,7	3,7		
	14,8	6,2	12,2	2,2	-	-	-	-	4,5	6,7	5,4	4,2		
camphor	7,2	3,4	3,4	3,7	-	-	-	-	4,1	2,9	1,9	2,0		
	9,5	3,9	8,2	1,3	-	-	-	-	2,5	4,0	3,2	2,4		
α-terpienol	1,9	-	-	-	-	-	-	-	-	-	-	-	-	
	2,3	-	2,0	-	-	-	-	-	-	-	-	-	-	
eugenol	7,0	-	-	-	-	-	-	-	-	-	-	-	-	
	7,9	-	4,6	0,8	-	-	-	-	-	-	-	-	-	
α-copaene	-	0,2	-	-	0,3	0,4	0,4	0,2	-	-	-	-	-	
	-	0,4	-	-	0,3	0,2	0,3	0,2	-	-	-	-	-	
β-elemene	1,6	-	-	-	1,0	0,9	2,2	0,8	-	-	-	-	0,4	
	0,9	-	1,1	-	0,7	0,8	1,0	1,7	-	-	-	-	0,4	
β-caryophyllene	10,1	4,5	6,8	2,0	6,4	6,9	8,2	4,7	-	-	-	-	-	1,8
	9,8	2,7	11,0	1,2	5,5	5,4	6,5	6,4	-	-	-	-	-	1,6
α-humulene	-	-	-	-	-	-	-	-	-	-	-	-	0,4	
	-	-	-	-	-	-	-	-	-	-	-	-	0,4	
germancrene B	2,4	0,8	1,4	0,3	1,3	1,5	1,7	1,0	-	-	-	-	-	
	2,1	0,4	2,3	0,3	1,1	1,0	1,3	1,4	-	-	-	-	-	
δ-cadinene	1,4	0,6	1,0	-	0,8	0,8	1,0	0,6	-	-	-	-	-	
	1,3	0,4	1,3	-	0,7	0,7	0,8	0,9	-	-	-	-	-	
germancrene-D-4-ol	1,7	0,9	1,4	0,3	1,5	1,4	1,7	1,0	-	-	-	-	-	
	0,8	0,3	0,9	0,3	1,1	1,1	1,3	1,2	-	-	-	-	-	
α-cadinol	8,4	0,8	-	-	1,2	1,6	1,6	1,2	-	-	-	-	-	
	7,3	-	8,7	1,3	1,1	1,3	1,6	1,5	-	-	-	-	-	

Source: Own authorship.

Variation in the chemical profile of the tea was observed in Table 2 according to the different extraction conditions. Two metabolites were detected in all the prepared teas - limonene and γ-terpinene, the latter present a relative abundance about 17 times higher than the other metabolites in all infusions. The literature reports some pharmacological properties of the limonene, such as: sedative, myorelaxant (Heinzmann and Barros 2007) antimicrobial on *Escherichia coli* and *Staphylococcus aureus* (Millezi et al. 2014). For the γ-terpinene, it is related to an antioxidant property (Freitas et al. 2015).

It was observed that the presence of the highest number of metabolites was associated with the use of fresh leaves and the condition that leads to obtaining the lowest number of metabolites was when dried leaves had been used. These data shows that, for basil, the drying process promotes a considerable loss of the most volatile secondary metabolites.

Cooled and frozen leaves (that were kept by 7 days, in the refrigerator and freezer) lead to the same chemical profile, evidencing that the storage of these leaves can be done in either refrigerator or freezer. In these cases, 5 metabolites were present: β-pinene, limonene, γ-terpineol, linalool, and camphor.

The β-pinene presents anti-inflammatory, antitumor, antioxidant and antimicrobial activities against the fungus *Candida albicans* and the bacteria *Staphylococcus aureus* (Silva et al. 2012). Linalool presents anti-inflammatory, anxiolytic,

sedative, anticonvulsant, vasorelaxant, hypotensive activities (Camargo and Vasconcelos 2014), antimicrobial against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Batista et al., 2019) and camphor shows antifungal activity against *Cryptococcus neoformans* and *Trichophyton mentagrophytes* (Santos et al. 2012).

The variation of the contact time between plant material and boiling water have not caused significant variations only to the infusions prepared from cooled and frozen leaves for 7 days, resulting in these cases, the same metabolites in relatively close proportions but, to the infusions prepared from dried leaves this infusion time variation caused significant variations in the chemical profile. For the, the contact between plant material and water for 5 minutes has led to the extraction of only 2 metabolites: limonene and γ -terpinene, while the extraction for 30 minutes has guaranteed the extraction of 5 metabolites: limonene, γ -terpinene, β -elemene, β -caryophyllene, and α -humulene. This data can be understood if we consider that, during the drying process, the most volatile and thermolabile metabolites must have been lost, so the heating promoted by the infusion process would only act to promote the migration of substances from the plant matrix to the aqueous medium. From a pharmacological point of view, a tea prepared with dried basil leaves that were infused for 5 minutes presented metabolites that have scientifically proven pharmacological effects: antimicrobial (Millezi et al. 2014) caused by limonene, and antioxidant (Freitas et al. 2015) caused by γ -terpinene. The tea that remained 30 minutes in infusion could, in addition to these effects, also present the metabolites responsible for the anticancer effect (Zhai et al. 2019) occasioned by β -elemene, antioxidant and antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida* sp. (Batista et al. 2019) occasioned by β -caryophyllene and anticancer activity (Legault and Pichette 2007) occasioned by α -humulene.

The chemical profile of the infusions prepared from the fragmented fresh leaves was different that was observed for the non-fragmented leaves, evidencing that the fragmentation favors the transfer of substances from the vegetable hue to the aqueous medium. Such favoring arises from the fact that, by fragmenting the plant tissue, cells are ruptured, favoring the extravasation of metabolites that are in the internal part of the cellular structure (Costa et al. 2005). Besides this, the fragmented plant material will present a larger contact surface with the solvent, facilitating the diffusion process (Oliveira et al. 2021).

For the fresh fragmented leaves infusions 14 metabolites were identified in the process where boiling water and fragmented leaves remained in contact for 20 minutes, 11 metabolites in the infusions with the contact was kept by 5 and 10 minutes, and 10 metabolites in the infusion where the permanence of the plant material in boiling water was for 30 minutes. This demonstrates that the contact time between the fragmented leaves and the hot water favors the extraction until the limit of 20 minutes; in this case, the favoring of metabolites migration to the solvent is the process that predominates. However, when the contact time between the plant material and boiling water reaches 30 minutes, the loss of those metabolites that are more volatile and/or thermolabile leads to the reduction in the number of metabolites present in the tea. Among all the metabolites extracted, the only ones present in the infusions prepared in the four different infusion periods were: β -caryophyllene, germacrene B, germacrene-D-4-ol, and α -cadinol. In the case of the infusions prepared with whole fresh leaves, the contact time between the plant material and the solvent have not changed the chemical profile, showing that the pharmacological properties of teas prepared with whole leaves tend to be more constant than those of teas prepared with fragmented leaves. Furthermore, among the infusions prepared with fresh leaves, only in those prepared by fragmented leaves the presence of the metabolites linalool, camphor and α -terpineol were observed. These metabolites exhibit anti-inflammatory, antidepressant, sedative, anticonvulsant, vasorelaxant, hypotensive and antimicrobial actions (Park et el. 2012; Santos et al. 2012; Camargo & Vasconcelos 2014; Batista et al. 2019).

4. Conclusion

The chemical profile of basil infusions experiences qualitative and quantitative variations according to the storage conditions of the plant material, as well as fragmentation of leaves and time in which the contact between the leaves and boiling water is kept during the infusion process.

The terpenes limonene and terpinene were the only ones detected in all extracts, regardless of the conditions of preparation and storage of the leaves. Limonene exhibited higher relative abundance than the others. This metabolite presents sedative, relaxing, and antimicrobial pharmacological actions.

The use of fresh fragmented leaves was the condition that has guaranteed the presence of the greatest variety of bioactive mono and sesquiterpenes in the teas (11 to 15 metabolites). Storing the leaves for 7 days both in the refrigerator and freezer have resulted in teas with the same chemical profile, evidencing that in home storage processes, both can be practiced. In this condition, teas with the same five metabolites were obtained, regardless of the contact time between the plant material and boiling water. For dried leaves, with an infusion time of 30 minutes, 5 metabolites were observed, but with an infusion time of five minutes, only two metabolites were detected in the teas, showing that when using dried basil leaves as raw material, the contact time between the plant material and the solvent should be longer if the objective is to obtain more bioactive metabolites.

The future perspective of our group is to continue the research by evaluating how the profile of terpenes in other herbal aromatic medicinal plant species is affected by these same storage and handling conditions. In addition, another goal is to investigate new extraction methodologies that are green, allowing for the efficient extraction of terpenes with biological properties that are present in medicinal plants.

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