ANALYSIS OF VOLATILE COMPOUNDS OF *Ilex paraguariensis* A. ST. - HIL. AND ITS MAIN ADULTERATING SPECIES *Ilex theizans* MART. EX REISSEK AND *Ilex dumosa* REISSEK

Análise de compostos voláteis de *Ilex paraguariensis* A. St. - Hil. e suas principais espécies adulterantes *Ilex theizans* Mart. ex Reissek e *Ilex dumosa* Reissek

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ABSTRACT

The adulteration of the product *Ilex paraguariensis* with other *Ilex* species is a mAjor problem for maté tea producers. In this work, three species of *Ilex* were evaluated for their volatile composition by headspace solid phase microextraction coupled to gas chromatography and mass spectrum detector (HS-SPME/GC-MS). The adulterating species *I. dumnosa* and *I. theizans* Mart. ex Reissek presented a different profile of volatile organic compounds when compared to *I. paraguariensis*. Aldehydes methyl-butanal, pentanal, hexanal, heptanal and nonanal were detected only in the adulterating species. This result suggests that such compounds are potential chemical markers for identification of adulteration and quality analysis of products based on *Ilex paraguariensis*.

Index terms: Food analysis, food composition.

RESUMO

A adulteração do produto *Ilex paraguariensis* com outras espécies de *Ilex* é um dos principais problemas dos produtores de erva-mate. Neste trabalho, três espécies de *Ilex* foram avaliadas quanto à sua composição volátil por microextração em fase sólida acoplada à cromatografia gasosa e detector de espectro de massas (HS-SPME/GC-MS). As espécies adulterantes *I. dumnosa* e *I. theizans* Mart. ex Reissek apresentaram um perfil diferente de compostos orgânicos voláteis, quando comparadas com a *I. paraguariensis*. Os aldeídos metil-butanal, pentanal, hexanal, heptanal e nonanal foram detectados apenas nas espécies adulterantes. Esse resultado sugere que esses compostos químicos são marcadores potenciais para a identificação de adulteração e análise da qualidade dos produtos à base de *Ilex paraguariensis*.

Termos para indexação: Análise de alimentos, composição de alimentos.

(Received in april 11, 2011 and approved in may 30, 2011)

INTRODUCTION

Maté tea (*Ilex paraguariensis* A. St.- Hil.) is a plant species native to South America. Its natural dispersion covers about 540,000 km^2, divided between the territories of Brazil, Argentina, Uruguay and Paraguay.

In Brazil, its dispersion area mainly covers the Southern region (RS, SC and PR), where it is routinely used in the preparation of maté, a stimulant drink, prepared by the infusion of its milled leaves and twigs. In 2002, Brazil produced about 500,000 tons of maté tea, and more than 48% was produced in Rio Grande do Sul (VALDUGA, 2002).

Maté undergoes a number of steps (parching, drying and roasting) during the production process that significantly influence the chemical composition of the final product and hence its sensory characteristics (VALDUGA, 2003; FILIP et al., 2010).

The maté market is often the victim of adulteration by substitution of *Ilex paraguariensis* leaves with other numerous plants of the same species and family, or even plants of other families. However, when added to maté, these plants modify the characteristics of the final product, and may cause health hazards to consumers (HECH et al., 2007).

Most plants used for adulteration belong to the species *Ilex*. The species *Ilex theizans* Mart. ex Reissek and *Ilex dumosa* Reissek belong to the genus *Ilex* and the family of Aquifoliaceae that are mostly used in adulteration of maté tea (FILIP et al., 1998; HECH et al., 2007; REGINATO et al, 1999). The identification of compounds present in maté (KAWAKAMI; KOBAYASHI, 1991; MACHADO et al., 2007; CANSIAN et al., 2008) and its adulterants (PIRES et al., 1997; FILIP et al., 1998) is the object of several studies.

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However, in most cases, the studies focus on identifying and/or quantification of terpenes (TAKETA et al., 2000 and 2004), saponins (GNOATTO et al., 2005; PIRES et al., 1997; REGINATTO et al., 1999), polyphenols (BRAVO et al, 2007), methylxanthines (FILIP et al, 1998 and 2010, REGINATTO et al, 1999) and flavonoids (TARRAGO et al., 2005, FILIP et al., 2010).

In a previous study, Pires et al. (1997) observed differences in the chemical composition of two species of *Ilex (dumosa and paraguariensis)*, especially related to the concentration of saponins. According to the authors, these differences could be used to identify adulterants in the maté tea. Caffeine is another compound that is found in significant quantities only in the species *Ilex paraguariensis*. In other species of this family caffeine only occurs at trace levels(FILIP et al., 1998; REGINATTO et al., 1999).

This work aimed to achieve a qualitative characterization of volatile compounds present in fresh and roasted samples of three different species of the genus *Ilex*. Compounds that can be used as chemical markers were identified. Such compounds would allow easy detection of adulteration in commercial maté, and also determination of the chemical transformations (responsible for the flavor of the product) that occur during processing.

MATERIAL AND METHODS

Samples

Samples of *Ilex paraguariensis* St.-Hil. were collected in the city of Barão de Cotegipe - RS. The samples of adulterants *Ilex theizans* Mart. ex Reissek and *Ilex dumosa* Reissek were previously cataloged (*Ilex dumosa* Reissek: record HFC - 6435, Latitude 25°17’ S Longitude 49°13' W, altitude: 950 m, collected on January 2009. *Ilex theizans* Mart. ex Reissek*:* report HFC - 6440, Latitude 25°17’ S Longitude 49° 13’ W Altitude: 950 m, collected in March 2009), and were supplied by the Brazilian Agricultural Research Corporation - EMBRAPA at the City of Colombo, State of Parana, Brazil.

The samples were randomly collected. Twenty kilograms of the species *Ilex paraguariensis* and 5 kg of the adulterant species were collected. The samples were placed in paper envelopes after collection and stored at approximately 10° C until extraction, which was carried out using 5 g of sample for each test.

A fraction of each sample was subjected to the process of roasting, as described by Valduga (2002). The purpose of this step was the denaturation of the enzymes present in the leaves and to decrease the water content.

Subsequently, the fresh and roasted samples were macerated, before passing through the extraction procedure, which was carried out in duplicate.

Conditions for isolation of volatile compounds

The extraction step was carried out by solid phase microextraction. The samples (5.0 g), previously macerated, were placed in a glass flask sealed with rubber septum. The polydimethylsiloxane fiber (100 mm, Supelco, USA) was previously conditioned following the instructions of the manufacturer. After conditioning the fiber was introduced into the flask and the temperature was gradually increased up to 60° C, and the fibers held at this temperature for an exposure period of 20 minutes. Subsequently, the fiber was collected and subjected to desorption in the gas chromatograph injector.

Instrumental Analysis

The samples were analyzed using a gas chromatograph coupled to a mass spectrum detector (GC-MS - Shimadzu, QP5050A, USA), operating at 1.2 kV impact energy. The fragments were collected between 40 and 400 Da. The chromatographic analysis was carried out using an initial temperature of 45° C, which was kept constant for 5 minutes, followed by an increase up to 190° C at 10° C min^{-1}, where it remained for more 10 minutes. The pressure used in the column was 25 kPa, with a flow rate of carrier gas (He) 0.7 mL min^{-1} and split ratio of 1 / 10. The chromatographic column used was a DB-5 (30m x 0.25 mm x 0.25 mm, J & W Scientific, USA), with nonpolar characteristics. The temperature employed in the injector, responsible for desorption of the compounds of the fiber, was 230° C. The interface temperature was 250° C. With the exception of linalool, acetic acid, ethanol, butanal, hexanal, propanoic acid and acetaldehyde, which were compared to external standards, the other compounds were identified by comparing their mass spectra with the database of the libraries of mass spectra (Wiley 229, Nist 21 and 107). In this case, these compounds were considered qualitatively identified.

RESULTS AND DISCUSSION

Compounds qualitatively identified using the libraries of mass spectra, with a probability (match quality) exceeding 90%, are presented in Table 1.

Table 1 – Volatile compounds qualitatively identified in fresh and roasted samples of the three species *Ilex*. The order of the compounds listed in the table corresponds to the order of elution.

Ilexp paraguariensis		*Ilex dumosa*		*Ilex theizans* Mart. ex Reissek	
fresh	*roasted*	*fresh*	*roasted*	*fresh*	*Roasted*
Acetaldehyde	Ethanol	2- methyl pentane	3-butanol	methyl ciclopentane	2- propanone
2-propanone	1-hydroxy, 2-propanone	methyl ciclopentane	Acetaldehyde	Octane	*3-methyl butanal*^{a}
Ethanol	Acetic acid	Butane	n-butane	butanal	*Pentanal*^{a}
2-hexenal	Octadecanal	Octane	trans-2,3 dimetil oxirane	2-methyl butanal	*1-pent-3-one*^{a}
1-hexanal	Hydroxydimethylester butanodioic acid	3-methyl butanal	*2-metil butanal*^{a}	3-methyl butanal	*Hexanal*^{a}
3-hexen-1-ol	H-pyrolene 2-carboxaldehyde	Ethanedienoic acid	3-methyl butanal	2,4-hexadienal	4- pentanal
2-hexen-1-ol	1,3 dihydroxy, 2-propanone	2- etil furane	butadienil acethylene	Ciclopentanol	*1-penten-3-ol*^{a}
Benzoic acid, 2-hydroxy metil esther	1-methylbutyl, hydroperoxide	Ciclopentanol	3 methyl- 2,4-hexadiene	Pentanal	*Heptanal*^{a}
Benzenemethanol	1,4-methyl, methylesther octadecanoic acid	Hexanal	*Pentanal*^{a}	Hexanal	Limonene
	2,3 dimethyl oxyrane, cis	Pentanal	2-propenoic acid	2-propanamine	3-methyl-1-butanol
	2-methyl benzaldehyde	n-pentanal	2,3 pentadiene	1-penten-3-ol	*Octanal*^{a}
	2-pirimidinamine	2-hexenal	*Hexanal*^{a}	2-hexanal	2,5-dimethylheptane
	4-hepten-3-one, 5-methyl	2-methyl-1-butanol	*Propanoic acid*^{a}	4-hexen-1-ol	2,7-dimethyl octane
	9,15,12 octadecatrienal	3-methyl - 1-butanol	*Heptanal*^{a}	2-pent-1-ol	6-methyl, 5-hepten-2-one
	2-heptanol, 5-ethyl	2-penten-1-ol	Ciclohexene	1-hexanol	*Nonanal*^{a}
	Undecanal	3-hexen-1-ol	2-methyl 1- butanol	3-hexen-1-ol	Dodecane
	Hexadecanoic acid	Nonanal	2-hexenal	2-hexen-1-ol	Acetic acid
	Caffeine	2-hexen-1-ol	*1-pentanol*^{a}	2-propen-1-ol, 2-metil	*2,4-heptadienal*^{a}
		1-octen-3-ol	*Octanal*^{a}	2,4-heptadienal	Butanal
		Linalool	Butyric, 2,2 dimethyl vinyl ester acid	2-methyl benzaldehyde	1-octanol
		1,2,15- pentadecanetriol	1-hexanol	ciclopentacicloheptene	2-methyl benzaldehyde
		1,1 oxibis octane	*3-hexen-1-ol*^{a}		2-butanol
		Naftalene	*Nonanal*^{a}		*4-penten-1-ol*^{a}
			Acetic acid		Propanodioc acid

[a] Compounds identified only in adulterants (roasted samples).

The sample of roasted *Ilex paraguariensis* presented an increase in the number of compounds extracted in relation with the fresh sample. This same behavior was also observed for the adulterants species studied (Table 1). Regardless of the species analyzed, significant reductions in the intensity (area) of peaks corresponding to the roasted sample were observed, when compared to the fresh samples. Both of these features (increase in the number of signals and reduction of their respective areas) have been linked to Maillard reactions (KAWAKAMI; KOBAYASHI, 1991) (presence of furfural in the roast sample of the *Ilex dumosa* species) and oxidative changes occurring during roasting. The high temperatures ($\cong$ 350° C) associated with the oxidizing atmosphere employed during roasting favors the oxidative cleavage of compounds of long carbon chains into compounds of smaller carbon chains, rather than the gradual oxidation of functional groups (alcohol $\rightarrow$ aldehyde $\rightarrow$ carboxylic acid) present in these compounds. Both changes lead to the formation of compounds with lower boiling points, favoring the volatilization and consequently its extraction. The presence of acetic acid only in the roasted sample of the *Ilex paraguariensis* species was linked to oxidative transformation of ethanol during the step of roasting.

Many alcohols and aldehydes qualitatively identified in the adulterant species (fresh and roasted) were observed by Kawakami and Kobayashi (1991) as volatile constituents of green and roasted maté.

Considering the percentage areas (Table 2) of the identified compounds, there was a predominance of alcohols and aldehydes in the three studied species (*Ilex paraguariensis, Ilex theizans* Mart. ex Reissek *and Ilex dumosa*). Among these two classes of compounds, a predominance of alcohols over aldehydes in the fresh samples was observed. Aldehydes prevail in the roasted samples.

This trend (aldehyde > alcohols) observed for the roasted samples was related with the conditions employed during the step of roasting (high temperature and oxidizing atmosphere), which favors the oxidation of alcohols to aldehydes and even to carboxylic acids. Similar results were observed by different authors studying green coffee and roasted coffee (MERRITT et al., 1985; SHIBAMOTO ; YEO, 1992; SHIBAMOTO; YAMAGUCHI, 1981).

The oxidative cleavage of the double bonds of fatty acids leads to the formation of aldehydes and ketones (VARNAM; SUTHERLAND, 1997). When lipids are exposed to heat in the presence of oxygen, they may decompose into secondary products, mainly aldehydes and alcohols (SHIBAMOTO; YAMAGUCHI, 1981).

The 2,4-heptadienal, a product of fatty acid degradation, was observed only for the roasted samples of *Ilex theizans* Mart. ex Reissek. This compound was also reported by Machado et al. (2007) and Kawakami and Kobayashi (1991) in the composition of volatiles in maté tea.

Some compounds identified in this study, such as linalool and hexanal, are employed by some authors to indentify teas of superior quality (SHIBAMOTO; KATO, 2001; GUYOT et al., 1982).

Other compounds were present only in the roasted samples of adulterant species (Table 1), like 5 to 8-carbon aldehydes. The presence of specific compounds in one or more species of the same family allows its distinction from the other species and can be used to certify the quality of a product.

Chemical indicators have been employed by Guyot et al. (1982) to differentiate a healthy green Arabica coffee from a coffee that was considered defective. The compounds ethyl isobutanoate, ethyl butanoate, ethyl 2-methyl-butanoate and n-hexyl acetate were observed only in the volatile fraction of the defective coffee. Similar studies were conducted by Merritt Junior et al. (1985) to identify

Table 2 – Contribution as a percentage of the area, linked to the compounds identified as alcohols and aldehydes present in fresh and roasted samples.

Species/sample	Alcohols (%)	Aldehydes (%)	$\sum$
Ilex theizans Mart. ex Reissek fresh	46.7	21.7	68.5
Ilex theizans Mart. ex Reissek roasted	2.8	46.8	49.6
Ilex dumosa Reissek fresh	42.8	11.3	54.2
Ilex dumosa Reissek roasted	11.2	43.8	55.0
Ilex paraguariensis fresh	95.0	2.8	97.8
Ilex paraguariensis roasted	13.5	6.3	19.8

certain types of coffee, considering the presence of some esters of high molecular weight alcohols, only found in Colombian coffee beans.

CONCLUSIONS

The proposed methodology was suitable to characterize, in a quick and simple manner, volatile compounds present in three species of *Ilex*, in fresh and roasted samples.

The qualitative differences observed between the volatile fractions of three species of *Ilex* allowed them to be differentiated from each other. The aldehydes methyl butanal, pentanal, hexanal, heptanal, octanal and nonanal were only observed in the adulterant species *I. theizans* Mart. ex Reissek and *I. dumosa*. They were defined as plausible chemical markers for use in the identification of adulterants during quality control of products derived from maté tea *I. paraguariensis*.

It is noteworthy that the conditions employed during the roasting stage (high temperatures and an oxidizing atmosphere) lead to oxidative transformations of the compounds present in *Ilex* samples, increasing the occurrence of aldehydes in relation to alcohols, what can improve the flavor of the final product.

ACKNOWLEDGEMENTS

The authors thank URI - Campus Erechim, FAPERGS and CNPq for financial support, Embrapa Forest - PR and Maté Tea Industry Barão for supplying the samples.

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