Plant Foods for Human Nutrition (2019) 74:358–363
https://doi.org/10.1007/s11130-019-00745-7

ORIGINAL PAPER

Volatile Compounds Determined by SPME-GC, Bioactive Compounds, *In Vitro* Antioxidant Capacity and Physicochemical Characteristics of Four Native Fruits from South America

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Published online: 17 June 2019

Abstract
The aim of the present study was to identify volatile organic compounds (VOCs) by SPME-GC and quantify the bioactive compounds (ascorbic acid, total flavonoids and total phenolic content), antioxidant capacity (DPPH and ORAC) and physicochemical characteristics of ocorocillo, cambucá, murici da praia and murici do campo, four native South American fruits. A total of 41 volatile compounds were identified in ocorocillo, of which 17 were terpenes. Cambuca's volatile profile contained aldehydes, aromatic hydrocarbons and alcohols. Murici da praia and murici do campo contained high levels of fatty acid volatiles and esters, that contribute to their remarkable aroma. Ocorocillo contained high levels of ascorbic acid and total flavonoids, while cambucá presented lower ascorbic acid, flavonoid and phenolic levels. Murici da praia and murici do campo contained high amounts of phenolic compounds and high free-radical scavenging capacity (DPPH and ORAC). In addition, this fruit was sweeter and less acid compared to the other assessed fruits. The results suggest that these native fruits constitute a good source of volatile compounds and bioactive compounds, which may aid in their preservation interest and potential use in the food, cosmetic and pharmaceutical industries.

Keywords Chemical composition · *Eugenia boliviana* · *Plinia edulis* · *Byrsonima stipulacea* · *Byrsonima crassifolia*

Introduction

In recent years, consumers have prioritized healthy foods and new sensory experiences, and knowledge on native genetic resources has attracted the attention of several economy sectors, due to peculiar flavors and characteristic aromas [1]. Contemporary human diets must not only provide the nutrients able to meet metabolic requirements, but also aid in promoting and improving health [2, 3]. Global interest in native fruits from South America has gradually increased due to the potential ability of their bioactive compounds to scavenge free radicals, as well as their anti-inflammatory, antimicrobial, and

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11130-019-00745-7) contains supplementary material, which is available to authorized users.

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anti-cancer activities. Recent studies have associated the dietary intake of high amounts of native fruits with lower cardiovascular disease, inflammation, and cancer incidences [4, 5].

Ocorocillo *(Eugenia boliviana*, Myrtaceae family*)* is a rare fruit tree originally found in Bolivia. It possess a thin sensitive skin, an intense orange color, and releases a unique characteristic aroma. Cambucá (*Plinia edulis*, Myrtaceae family) is a fruit tree originally found in the Brazilian Atlantic Rainforest, attractive due to its yellow-orange pulp and sweet-acidic flavor, but almost extinct due to the deforestation of this biome. Murici da praia (*Byrsonima stipulacea,* Malpighiacaeae family) and murici do campo (*Byrsonima crassifolia*, Malpighiacaeae family) are fruit trees found in all Brazilian biomes. Murici da praia can grow from 2.5 to 10–20 m depending on the region, forming a pyramidal crown, while murici do campo is a small tree ranging from 2 to 4 m in height. Fruits from both species are yellow, sweet, slightly acidulated with their skin adhered to the pulp. Their pulp has an oily appearance and can be used to prepare jellies and ice creams.

Volatile compounds are substances released by foods that confer aroma and influence taste [6], directly affecting consumer buying decisions. They emerge from a complex blend of secondary metabolites, including terpenes, esters, aldehydes, ketones, and alcohols [7]. Terpenes, especially sesquiterpenes, display analgesic and antimicrobial properties. Moreover, these compounds also protect against degenerative diseases of the central nervous system and inhibit cell proliferation in breast and colon cancers [8, 9].

Studies have reported bioactive compounds and antioxidant capacity in native fruits from South America [10–12]. However, few studies have addressed the organic volatile compound profile in murici do campo [6, 13] and no studies have reported organic volatile compounds in ocorocillo, cambucá, and murici da praia. Compound characterization and quantification in ocorocillo, cambucá, murici da praia, and murici do campo provide unprecedented information, useful for their preservation and of interest to the food, pharmaceutical and cosmetic industries. In this context, SPME/GC-MS was used to identify volatile compounds in these fruits, while bioactive compounds concentrations, including ascorbic acid content, total flavonoid content, and total phenolic content, were also determined. Finally, the free-radical scavenging capacity (DPPH and ORAC) and physicochemical characteristics for the four fruits were also assessed.

Material and Methods

The material and methods section is presented as Supplementary Material 1.

Results and Discussion

SPME/GC-MS Analysis of Volatile Organic Compounds (VOCs)

The volatile organic compounds identified in ocorocillo, cambucá, murici da praia and murici do campo fruit are displayed in Table 1 and Fig. 1 (Supplementary Material 1).

A total of 41 compounds were identified in ocorocillo, including five monoterpenes, 12 sesquiterpenes, 14 esters, six alcohols, two aliphatic alcohols and aldehydes, one aldehyde and one ketone. Sesquiterpenes and monoterpenes accounted for 52.9% of the total pulp VOCs, whereas esters represented 21.33%, alcohols, 13.82%, other alcohols and aldehydes, 12.09% and ketones, 0.45%. These compounds are responsible for a woody aroma and fruity flavor.

Sesquiterpenes β-caryophyllene (CAR), germacrene D, and α-humulene (HUM) represented 17.92, 5.12 and 4.71%, respectively. Monoterpenes and sesquiterpenes are natural aromatic compounds found in condiments and essential oils, often used in alternative medicine drugs and in drugs developed by the pharmaceutical/cosmetic industry [8]. Ojha et al. [9] analyzed the neuroprotective effect of CAR against rotenone-induced oxidative stress and neuro-inflammation in a Parkinson’s disease at model. Their results indicated that CAR inhibits the production of pro-inflammatory cytokines and inflammatory mediators, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Nguyen et al. [8] tested the potential effects of CAR, β-caryophyllene oxyde (CAO) and HUM on the key drug-metabolizing enzymes activities, and their results indicate that these sesquiterpenes inhibit cytochrome P450 3A activity in human liver.

Fourteen volatile compounds were identified in cambucá pulp, mainly aldehydes (33.33%), aromatic hydrocarbons (14.32%), and, alcohols (9%) (Table 1 and Fig. 1, Supplementary Material 1). Toluene, hexanal and 3-acetyl-2,5-dimethyl were the most representative. Cambucá can be described as sweet and pungent, with fruity, and nut-like notes. The VOCs analysis also revealed the presence of monoterpenes, alkanes, alkenes, ketones, furans, benzoic acid, and esters. Dissimilar to cambucá, sapodilla (*Manilkara sapota* L.) an America fruit grown in the Brazilian Amazon volatile compounds consist mainly of esters and alcohols [13].

Hexanoic acid (a volatile fatty acid*)* and 2-pentanone (a ketone) are responsible for 81.84% of all VOCs identified in murici da praia (Table 1 and Fig. 1, Supplementary Material 1). Cheesy and goat-like notes in this fruit contribute to its aroma. Hexanoic acid (a volatile fatty acid) and ethyl hexanoate (a fatty acid ethyl ester) correspond to 90.09% of the murici do campo aroma, associated with cheesy, goat-like and fruity pineapple notes. Fatty acids belong to the lipid metabolism, and have been linked to fatty, cheese and rancid attributes [14, 15]. Some fatty acids are effective in reducing the risks for cardiovascular disease, cancer, osteoporosis and diabetes [15, 16]. The results described herein agree with those reported by Alves and Franco [6], which state that the characteristic murici aroma (*Byrsonima crassifolia*) is related to fatty acids. The same authors reported at predominant compounds were esters, followed by alcohols. Uekane et al. [13] reported that the murici (*Byrsonima crassifolia)* volatile fraction contains high amounts of esters, followed by carboxylic acids. Both studies are similar to those described herein.

Volatile organic compound composition and levels vary with species, methodology, growing region and fruit maturation. Murici present an unusual aroma, and may not be accepted in some fresh markets, but may still serve for the food processing industry and be useful in cosmetic formulations. To the best of our knowledge, no reports concerning VOCs in *Byrsonima stipulacea* are available.

Bioactive Compounds

Table 2 and Fig. 2 (Supplementary Material 1) present ascorbic acid, total flavonoid and total phenolic contents for the four assessed fruits.

Ascorbic acid concentrations were 18.14 mg 100 g^{-1} fresh weight (FW) for cambucá and 44.93 mg 100 g^{-1} FW for ocorocillo. The ascorbic acid content for murici da praia and murici do campo was not determined. The consumption of 200 g of cambucá and ocorocillo contributes to 40.30–88.84 and 48.36–119.8% of the Recommended Dietary Allowances (RDA) of ascorbic acid content for male and female adults, respectively [17]. Ocorocillo contains ascorbic acid levels similar to those in vitamin C-rich citric fruits (orange, grapefruit, tangerine, lemon), native Brazilian fruits (araçá, buriti, cagaita) and native Colombian fruits (abiu, cajá, and papaya) [10–12, 18].

The assessed native fruits differed in total flavonoid contents ($P < 0.05$). Ocorocillo and murici da praia contained higher levels, murici do campo fruit contained intermediate concentrations and cambucá, the lowest levels (Table 2 and Fig. 2, Supplementary Material 1). Flavonoids are considered important biological active compounds with high antioxidant potential and numerous health benefits [19]. Haminiuk et al. [20] analyzed the chemical composition of Brazilian fruits and determined flavonoid levels in grumixama, cambuci, and jabuticaba at 14.87, 30.16 mg and 31.60 mg 100 g^{-1} FW, respectively. These fruits are considered promising Brazilian native fruits, interesting as fresh products due to their high biological activity, while their distinctive flavor and aroma when processed is interesting for different industries.

A significant variation in total phenolic compound contents was observed (118.83 to 457.63 mg GAE 100 g^{-1} FW) (Table 2 and Fig. 2, Supplementary Material 1), with murici do campo containing 3.85-fold higher phenolic compound levels than cambucá. These values are higher than those reported for other fruits, such as passion fruit (61 mg GAE 100 g^{-1} FW), mango (60 mg GAE 100 g^{-1} FW), pear apple (40.8 mg GAE 100 g^{-1} FW) and mountain papaya (36.8 mg GAE 100 g^{-1} FW) [10, 11]. Schiassi et al. [12] analyzed araçá, buriti, cagaita, cajá, mangaba and marolo pulps (all native Brazilian Cerrado fruits), and observed that marolo was the only fruit to present higher total phenolic concentrations (728.17 mg 100 g^{-1} FW). da Silva et al. [21] reported total phenolic compound contents of 380 and 430 mg 100 g^{-1} FW for two jabuticaba species, native Brazilian Atlantic rainforest fruits. Phenolic compounds are a large class of plant compounds, considered responsible for the antioxidant capacity of fruits and vegetables [19, 20].

Antioxidant Capacity

The antioxidant capacity of the four assessed fruits was investigated by the DPPH (2,2- diphenyl-1-picrylhydrazyl) and ORAC (oxygen radical absorbance capacity) assays. The results are displayed in Table 2 and Fig. 2 (Supplementary Material 1).

Murici do campo and murici da praia displayed the highest antioxidant capacity in the ORAC assay (2,318.02 and 2,074.12 μmol trolox g^{-1} DW, respectively) compared to the other investigated fruits. The same behavior was verified for the DPPH assay, where values of 1,588.35 and 501.78 μmol trolox g^{-1} DW were determined for the murici do campo and murici da praia, respectively.

The high antioxidant capacity of the murici fruits compared to the other assessed fruits may be due to the higher quantified phenolic compound levels, mainly quercetin, which is considered one of the most efficient free radical scavengers [5]. With the exception of cambucá, the other fruits reported herein displayed higher antioxidant capacity levels than those cited by Tiveron et al. [22], when analyzing the antioxidant capacity of 22 species of commonly consumed vegetables in Brazil. Those authors reported a range from 2.3 to 70.1 μmol trolox g^{-1} DW for cucumber and artichoke, respectively. In turn, cambucá presented higher antioxidant activity than 15 plants described by Tiveron et al. [22].

Correlation between Bioactive Compounds and Antioxidant Capacity

Table 3 (Supplementary Material 1) displays Pearson's linear correlation among the bioactive compounds present in the assessed native Brazilian fruits. Total flavonoids in ocorocillo displayed a highly negative correlation with total phenolic content. On the other hand, phenolic compounds were positively correlated to the ORAC and DPPH assay methods. This indicates that phenolic compounds are one of the main components responsible for the antioxidant behavior in ocorocillo, independent of the detection method. A positive linear correlation between total antioxidant capacity, determined by the FRAP method, and phenolic content also was observed by Bertoncelj et al. [23].

In cambucá, total flavonoids were positively correlated to DPPH (Table 3, Supplementary Material 1). The antioxidant capacity determined by the ORAC assay presented a negative correlation with total flavonoids and phenolic compounds. A moderately positive correlation was noted between the DPPH method and total flavonoid content, but a negative correlation was noted between ORAC and flavonoids and phenolics. The same ORAC behavior was observed in the 50 most popular antioxidant-rich US foods [24]. For both murici da praia and murici do campo, total flavonoids were negatively correlated with phenolic compounds, while displaying a positive correlation with ORAC. Phenolic compounds presented a negative correlation with ORAC. Phenolic compounds were only positively correlated to the DPPH assay method in murici da praia. The reaction mechanisms underlying each method may explain these results. Whereas ORAC depends on the transfer of hydrogen atoms, the DPPH test relies on electron transfers [25]. Assays based on the hydrogen transfer act on more common physiological radicals, in contrast with electron transfer assays [26].

According to these results, total phenolic content promotes the antioxidant capacity mediated by hydrogen atom transfers and electron transfer in ocorocillo, cambucá and murici da praia, respectively, while total flavonoids promote most of the antioxidant capacity by hydrogen atom transfers in murici da praia and murici do campo (Table 3, Supplementary Material 1).

Physicochemical Characteristics

The physicochemical characteristics of native fruits are presented in Table 4 (Supplementary Material 1).

Cambucá were larger in size and displayed greater fresh mass than the other fruits ($P < 0.05$). However, this fruit presented a lower pulp yield (35.49%) compared to murici do campo (82.31%) and ocorocillo (80.30%). Cambucá, murici da praia and murici do campo can be described as yellow in color (L* = 79.12, 69.35 and 70.45; °h = 83.79, 85 and 84.39, respectively), while ocorocillo is yellow-orange (L* = 53.21 and °h = 60.03). Cambucá presented a less intense color (7.4) than the other assessed fruits, as indicated by chromaticity values (C) (Table 4, Supplementary Material 1).

Murici do campo presented higher total soluble solids (TSS) values ($P < 0.05$) at 13.2 °Brix. Murici da praia showed intermediate levels (9.7 °Brix), followed by cambucá (7.5 °Brix) and ocorocillo (5.65 °Brix). Morzelle et al. [27] reported 12.06, 11.70 and 11.83 °Brix for curriola (*Pouteria ramiflora*), gabiroba (*Campomanesia cambessedeana*) and murici (*Byrsonima verbascifolia*), respectively. For TTA, cambucá presented 1.38 g citric acid 100 g^{-1} FW, ocorocillo contained 0.80 g citric acid 100 g^{-1} FW, 0.31 g citric acid 100 g^{-1} FW were determined in murici do campo and 0.30 g citric acid 100 g^{-1} FW was observed in murici da praia.

Murici da praia and murici do campo displayed a high TSS/TTA ratio (Table 4, Supplementary Material 1), following by ocorocillo and cambucá. Although acidity levels are high in cambucá, this fruit is not characterized as acidic, due to the sugar/acid ratio. On the other hand, sugar levels in murici da praia and murici do campo are higher, but their perception is not equivalent, due to the high concentrations of fatty acids in this fruit. Murici do campo pH was higher than the other analyzed fruits ($P < 0.05$) (4.42). Uvaia (*Eugenia pyriformis*) pH is lower than reported herein [28], however is similar to gabiroba (*Campomanesia cambessedeana*) and murici (*Byrsonima verbascifolia*) native Brazilian cerrado fruits [27]. The physicochemical characteristics of each fruit should be taken into account for both *in natura* consumption and industrial processing [28].

Conclusions

The results reported herein indicate that terpene content in ocorocillo, including β-caryophyllene, germacrene D and α-humulene, confer a peculiar fruity and woody aroma. This fruit is a good source of ascorbic acid and phenolic compounds, and displays high antioxidant capacity, moderate acidity and low sweetness. Toluene, hexanal and 3-acetyl-2,5-dimethyl are the most representative VOCs in cambucá which, despite being reported as a tasty fruit, contains lower levels of ascorbic acid, flavonoids and phenolics, but higher levels than traditional fruits, which are highly cultivated and consumed. In addition, this fruit presented high acidity and moderate sweetness.

The most representative VOCs identified in murici do campo were hexanoic acid (a volatile fatty acid) and ethyl hexanoate (a fatty acid ethyl ester), which are responsible for the aroma in this fruit, while hexanoic acid (volatile fatty acid) and 2-pentanone (ketone) were identified in murici da praia. Both murici do campo and murici da praia contained high levels of total flavonoids and phenolic compounds, as well as high antioxidant capacity determined by the DPPH and ORAC methods. In addition, they are are sweeter and presented lower acidity.

These results suggest that native South America fruits are a good source of interesting volatile compounds and high levels of bioactive compounds, and may present different uses as ingredients in the food industry, as well as in the cosmetic and pharmaceutical industries.

Acknowledgements The authors would like to thank grants #2014/13473-7 and #2013/07914-8, São Paulo Research Foundation (FAPESP), Coordination for the Improvement of Higher Education Personnel (CAPES), and the National Council for Scientific and Technological Development (CNPq, research productivity grant #308521/2015-3 and the research funding grant #458123/2014-5), which provided financial support and a scholarship for the development of this study. The authors are also grateful to Sérgio Sartori and Helton Muniz, Rio Claro and Campina do Monte Alegre, São Paulo, fruit farmers for providing the fruits analyzed in the present study.

Compliance with Ethical Standards

Conflict of Interest All authors declare they have no conflict of interest. This article does not contain any studies with human or animal subjects.

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