Evolution of aroma compounds of murtilla fruits (*Ugni molinae* Turcz) during storage

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Abstract

BACKGROUND: 'Murtilla', 'mutilla' or 'murta' (*Ugni molinae* Turcz) is a native Chilean species that produces a small berry fruit with a special aroma, whose volatile compounds have not yet been identified. The fruit may be consumed raw and also as jams, juice, canned products, confections and liquor.

RESULTS: At the beginning and end of the storage, 24 volatile compounds were identified in murtilla fruit aroma and the concentration of these compounds in murtilla fruit ranged from 1.2 to 250.5 μg kg^{-1} fresh weight. Methyl 2-methyl butanoate, ethyl butanoate, ethyl 2-methyl butanoate, methyl hexanoate, ethyl hexanoate, methyl benzoate and ethyl benzoate were the major components, all of which have been reported as potent odors in other aromatic fruits. Based on estimated odor activity value, the most potent compound in the murtilla fruit aroma were ethyl hexanoate and 4-methoxy-2,5-dimethyl-furan-3-one. The statistical analysis showed that the storage produced a distinct effect on the same volatile compounds released from the murtilla ecotypes.

CONCLUSION: The volatile compounds identified in murtilla fruit aroma, which may be described as fruity, sweet and floral, have been found in other aromatic fruits. Concerning the aroma, the murtilla fruit from ecotype 19-1 was shown to be the best in cooled storage.

Keywords: murtilla; *Ugni molinae* Turcz; volatile compounds; aroma; storage; GC–MS

INTRODUCTION

'Murtilla', 'mutilla' or 'murta' (*Ugni molinae* Turcz) is a native Chilean species belonging to the Myrthaceae family. This plant is a wild perennial shrub growing in the south of Chile (between the VII and XI regions), mainly in the coastal mountains and some places in the Andean mountains.[1–4]

The murtilla plant produces a small globoid berry fruit with an equatorial diameter of 0.7–1.3 cm. Different plant ecotypes develop a variety of fruit colors including soft green, yellow, fuchsia (purplish), light and dark red.[5] The ripe murtilla fruit exhibits a special and surrounding aroma, although the volatile compounds have not yet been identified. Murtilla is consumed as a fresh fruit because of its organoleptic characteristics, although it is also processed by the food industry for making jam, juice, canned items, confections and liquor products.

Chemical parameters that contribute to the organoleptic characteristics of murtilla fruit have been reported. Fruits collected in southern Chile showed high variability in the soluble solids ranging from 6.5 to 29°Brix and pH ranging between 4.7 and 5.2.[5,6] The titratable acidity (6.9–8.4 mEq NaOH), and the content of fructose (2.42–4.07 g), glucose (1.45–1.88 g) and sucrose (3.43–4.95 g) were measured in 100 g of fresh weight murtilla fruits.[7]

The distinct aroma plays an important role in the distribution of murtilla in the fruit market and flavor industries. Therefore, researchers are interested in determining the volatile constituents of the aroma in order to identify the key compounds and to evaluate the effect of both the post-harvest storage and processing on aroma composition.

There is no more information about murtilla plant chemical characteristic, but recently leaves chemical

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components with antioxidant and anti-inflammatory activities have been reported.[8–10]

In Chile the murtilla fruit used in the food industry or for fresh consumption are primarily of wild origin. For this reason the Instituto de Investigaciones Agropecuarias (INIA-Carillanca, Chile) has instituted a program to domesticate this plant. Early results obtained by the project FDI-CORFO N° 02C8AT-04 have allowed the selection of several ecotypes with agronomical potential. In this scope, the objective of this study was to identify the main volatile compounds of the murtilla fruit aroma originating from promissory ecotype plants and to evaluate their stability in cooled storage.

EXPERIMENTAL

Raw material

The fruit was obtained from four murtilla (*Ungi molinae* Turcz) ecotypes (14-4, 17-2, 19-1 and 33-5; INIA Carillanca Murtilla Gene Bank) growing in an experimental field near to Puerto Saavedra (latitude, 38° 45′ S; longitude, 73° 21′ W).

Because the experimental field is located in a coastal area near to the Pacific Ocean, the weather is characterized as being a moderate oceanic climate with marine influence. Fruit from 2.5-year-old murtilla plants, which were drip irrigated periodically during spring and summer, were used in the experiment.

The ecotypes used in this research were selected on agronomical and organoleptic characteristics. The 14-4 and 19-1 plant ecotypes are characterized by high fruit yield whereas the fruit from 17-2 and 33-5 ecotypes present a yellow and pink color, respectively, that are different from the typical murtilla color.

The fruit was harvested on the 10 April 2006 and immediately carried to the Food Science Laboratory of the Universidad de La Frontera. Fruits with a diameter of approximately 0.9 cm were packaged, slowly cooled and stored for 60 days at 0 °C in a chamber (Archiclima Ltda., Chile). The fruits that were immature, over-ripe or damaged were discarded. Sixty days of storage was chosen as this is the average time of storage between harvesting and reaching the final consumer.

Experimental design

The volatile compounds from the aroma of the murtilla fruit were evaluated initially and after 60 days of storage at 0 °C. Approximately 100 g of the fruit were packed in a polyethylene terephthalate clamshell punnet with 24 ventilation holes (Typack S.A., Chile). Evaluation of the volatile compounds was carried out with two replicates for each murtilla fruit ecotype (14-4, 17-2, 19-1 and 33-5) at the beginning and at the end of the experimentation period.

Volatile compounds trapping procedure

Airborne volatiles of murtilla fruit were trapped following the methodology reported by Franco and Shibamoto[11] and Quiroz *et al.*[12,13] A column with 100 mg of Porapak Q (80–100 mesh, Waters Associates Inc., USA) was used for trapping volatile compounds. Porapak Q was cleaned with 750 μL of diethyl ether (GC, grade Merck, Germany) and then heated at 150 °C for 2 h with a nitrogen (99,995%, AGA-Chile) flow at 70 mL min^{-1}.

Murtilla fruit (300 ± 1 g) was deposited in a 900 mL bell jar (6 cm internal diameter, 30 cm high). Air was dried and purified by passage through activated 5 Å molecular sieves, and then charcoal, and finally drawn for 20 h at 1 L min^{-1} through two bell jars containing murtilla fruits. Volatiles were absorbed onto Porapak Q inside containers placed on the outlets of each bell jar; volatiles were extracted by elution with 750 μL of fresh hexane (GC–MS grade, Optima, Fisher Scientific, Fair Lawn, New Jersey, USA).[11–13]

Analysis of volatile compounds by GC–MS

The volatile compounds were analyzed using a gas chromatograph (Model Focus, Thermo Electron Corporation, Waltham, USA) coupled to a mass spectrometer (Model DSQ, Thermo Electron Corporation) equipped with a HP-Ultra 1 capillary column (25 m, 0.2 mm, 0.33 μm). Helium was used as the gas carrier, with a flow rate of 1.5 mL min^{-1}. Mass spectrum acquisition was performed in the mass range from 35 to 500 m/z. Ionization was performed by electron impact at 70 eV with an ion source at 200 °C. A 2.0 μL sample in splitless mode was injected for each sample with the injector temperature of 250 °C. The oven temperature was programmed to remain at 40 °C for 1 min and then increased 5 °C min^{-1} to 260 °C and held for 5 min. The interface temperature was programmed at 250 °C. Volatiles were identified by comparing GLC Kovats indices (KIs) and mass spectra (MS) with the corresponding commercial pure standards (Sigma Aldrich). The experimental KIs were compared with theoretical KIs from pure standard compounds and reported in the Flavornet database.[14] Resulting spectra were compared with a library database by a reverse search technique.

Calibration curves based on peak area ratio were constructed using pure standards and docosane as an internal standard for quantification of each volatile compound identified in murtilla fruit aroma.

Statistical analysis

An analysis of variance (StatMost 3.0) at 5% significance level was used to analyze the influence of the ecotype and storage time on quantitative result of the volatile compounds. The difference between means of quantitative date of each volatile compound was compared using Duncan's multiple range test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Aromatic volatile compounds of the murtilla fruit

Volatile compounds identified by GC–MS analysis from four different ecotypes of the murtilla fruit are

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shown in Table 1. These compounds correspond to the headspace volatile fraction from intact murtilla fruits that were absorbed onto Porapak Q. The trapped volatiles were eluted with hexane and analyzed by GC–MS.[11–13] The concentrations showed in Table 1 correspond to volatile compounds in headspace referred to weight fresh of the murtilla fruit in a raw nature. Table 2 shows the odor characteristic and method used for identification of the volatile compounds of the murtilla fruit aroma.

This study was centered on the major and common volatile compounds between ecotypes evaluated, which are typical in the volatile composition of other fruit aromas.

At the initial of the storage, methyl hexanoate presented the major concentration among volatile compounds identified for murtilla fruit from ecotype 14-4 (223 $\mu g\,kg^{-1}$ fresh weight), 17-2 (250 $\mu g\,kg^{-1}$ fresh weight) and 33-5 (147 $\mu g\,kg^{-1}$ fresh weight), whereas methyl benzoate showed the highest concentration (158 $\mu g\,kg^{-1}$ fresh weight) for murtilla fruit aroma obtained from ecotype 19-1 plants (Table 1). Methyl 2-methyl butanoate, ethyl butanoate, methyl pentanoate, ethyl 2-methyl butanoate, methyl hexanoate, α-pinene, ethyl hexanoate, 1,8-cineole, D-limonene and methyl benzoate are common volatile constituents found in the four ecotypes fruit, but their concentration differs.

All the compounds listed in Table 1 are frequently found in aroma compositions of several fruits and spices. Ten of them (ID 2, 3, 4, 7, 8, 10, 12, 15, 18 and 19 in Table 1) have been found in the freshness flavor of strawberries.[15,16] The sensory evaluation showed that strawberry fruit is always highly appreciated if its sweetness and aroma intensity are high. Methyl butanoate, ethyl butanoate, methyl hexanoate, *cis*-3-hexenyl acetate and hexyl acetate were the compounds which contributed to this overall appreciation of strawberry quality.[15] Methyl hexanoate was the major volatile constituent (147–250 $\mu g\,kg^{-1}$ fresh weight) determined in the aroma of three murtilla fruit ecotypes at the initial of the storage. This compound was found in high concentration (3.12–9.02 $mg\,kg^{-1}$) in the ripe strawberry fruit varieties Carezza, Darselect and Marmolada.[16] Ethyl butanoate, butyl acetate, methyl hexanoate, ethyl hexanoate and hexyl acetate identified in murtilla fruit aroma have been considered as important volatile compounds of the strawberry aroma and were selected as indicators to evaluate the effect of the storage temperature on the aroma evolution during post-harvest.[17]

Butyl acetate, ethyl 2-methyl butanoate, ethyl hexanoate, hexyl acetate, α-pinene and limonene (Table 1) have been identified in the volatile composition of the 'Marion' and 'Thornless evergreen' blackberries, but their proportion is low in both cultivar, except α-pinene in 'Thornless evergreen'.[18]

Several compounds found in the murtilla fruit aroma of the four ecotypes also are present in the volatile composition of the tropical fruits pitanga (*Eugenia uniflora* L.), umbu-caja (*Spondias citherea*), camu-camu (*Myrciaria dubia*), araça-boi (*Eugenia stipitata*), cupuaçu (*Theobroma grandiflorum*), murici (*Byrsonima crassifolia* L., Rich), cashew apple (*Anacardium occidentale* L.) and mango fruit.[11,19–22] Considering that the terpenes are the predominate compounds in some of these fruits, two esters, ethyl butanoate (42%) and ethyl hexanoate (22%), were the major constituents identified in camu-camu (*Myrciaria dubia*) samples.[11] The esters were predominate compounds in murici (*Byrsonima crassifolia* L., Rich), specifically ethyl hexanoate (25.1%) and methyl hexanoate (5.1%).[20] Others volatile ester compounds like ethyl 2-methyl propanoate, methyl and ethyl pentanoate, ethyl (2*E*)-but-2-enoate, ethyl 2-methyl butanoate, ethyl 3-methyl butanoate, methyl and ethyl benzoate determinate for murtilla fruit (Table 1) have been reported in the aroma composition of the tropical fruits.[23] According to these authors, tropical fruits have called the attention from the aroma industries, because their flavor and aroma, frequently considered as exotic, is a characteristic that stimulate their consumption worldwide. Moreover, butyl acetate, ethyl 2-methyl butanoate, ethyl hexanoate, hexyl acetate methyl octanoate, ethyl benzoate, limonene and benzaldehyde (Table 1) are volatile constituents reported in the flavor or aroma of traditional fruits (apple, apricot, peach and pear) in a raw state such as in a juice or nectar product.[24–26] These results show that murtilla aroma is a mixture of tropical and traditional aroma constituents.

Differences in both aroma volatile composition and concentration were observed among the four ecotypes of murtilla fruit at the initial of the storage (Table 1). Six common volatile compounds (2, 3, 8, 12, 15 and 20) showed the higher concentrations in the ecotypes evaluated. A significant difference ($P < 0.05$) was observed when it was compared the concentrations of these compounds among the ecotypes. Similar results have been reported for strawberry and apricot, showing that different varieties show qualitative and quantitative differences in aroma composition.[16,26]

The high standard deviations observed in Table 1 could be attributed to differences in fruit maturation, which was not evident when samples were selected by size and general appearance. High standard deviations will need to be clarified in future studies in which the fruit ripening process will be evaluate by soluble solids, pH, firmness, color and other parameters and then correlated with volatile composition in each fruit maturity stage.

Potentially important volatile compounds detected in murtilla fruit aroma

The odor activity value (OAV) was estimated for analyzing the potential importance of the volatile compounds for aroma released by the four ecotypes of murtilla fruit at the initial and end of the storage.

Table 3 show the volatile compounds (identify by ID, as in Table 1) with higher odor activity

Table 1. Concentration (μg kg^{-1} fresh weight) of the aroma volatile compounds released from four ecotypes of the murtilla fruit at the initial and end of the storage at 0 °C for 60 days

		Ecotype 14-4[a]		Ecotype 17-2[a]		Ecotype 19-1[a]		Ecotype 33-5[a]	
Volatile compound[c]	ID	Initial[b]	End[b]	Initial[b]	End[b]	Initial[b]	End[b]	Initial[b]	End[b]
Methyl butanoate	A	ND	16.7[a] ± 21.2	ND	ND	ND	12.8a ± 13.1	ND	ND
Ethyl 2-methyl propanoate	1	ND	9.5[a] ± 5.5	4.5[a] ± 2.3	6.1[a] ± 0.3	4.7[a] ± 0.0	7.3[a] ± 5.2	3.4[a] ± 0.2	2.6[a] ± 0.0
Methyl 2-methyl butanoate	2	123.2[a] ± 2.1	23.7[c] ± 11.0	127.1[a] ± 25.7	15.8[c] ± 0.3	73.3[b] ± 0.4	8.6c	80.5[b] ± 8.8	5,2[c] ± 2.3
Ethyl butanoate	3	20.8[b] ± 0.5	153.6[a] ± 84.4	161.6[a] ± 60.0	90.7[ab] ± 0.5	143.2[a] ± 0.4	121.5[ab] ± 89.1	41.3[ab] ± 4.8	42.8[ab] ± 1.2
n-Butyl acetate	4	ND	4.4ab	7.3[a] ± 3.7	9.0[a] ± 0.5	ND	ND	1.3[b] ± 0.1	ND
Methyl pentanoate	5	19.9[a] ± 1.0	4.8c	12.3[b] ± 0.8	1.2[c] ± 0.1	6.9[c] ± 0.3	2.0[c] ± 2.7	9.7[b] ± 0.8	ND
Ethyl (2*E*)-but-2-enoate	6	ND	ND	27.6[a] ± 4.5	21.6[ab] ± 1.6	6.3b[c] ± 0.0	15.1[abc] ± 12.1	2.3[c] ± 0.4	ND
Propan-2-yl butanoate	7	8.3[a] ± 1.7	ND	6.4[a] ± 0.5	ND	ND	ND	1.7[b] ± 0.2	ND
Ethyl 2-methyl butanoate	8	4.1[c] ± 0.5	89.4[a] ± 33.7	80.0[ab] ± 29.2	34.0b[c] ± 2.0	50.0[abc] ± 3.5	71.6[ab] ± 31.7	11.7[c] ± 1.9	13.8[c] ± 0.7
Ethyl 3-methyl butanoate	9	ND	ND	38.5[a] ± 5.9	12.7[b] ± 0.2	13.0[b] ± 1.3	ND	4.0[c] ± 0.5	ND
3-Methylbutyl acetate	10	ND	ND	3.9[b] ± 0.5	6.7[a] ± 0.5	ND	ND	ND	ND
Ethyl pentanoate	11	ND	8.9[a] ± 2.9	6.3[ab] ± 1.6	4.0bc ± 0.4	6.9[ab] ± 0.3	7.5[ab] ± 3.2	1.1[c] ± 0.1	1.0[c] ± 0.2
Methyl hexanoate	12	223.5[a] ± 33.2	121.8[ab] ± 138.4	250.5[a] ± 20.3	48.9[b] ± 4.8	97.2[ab] ± 8.9	97.0[ab] ± 110.4	147.5[ab] ± 26.2	23.9[b] ± 0.9
Benzaldehyde	13	9.1[ab] ± 1.1	ND	ND	5.3c	8.2[b] ± 0.3	9.6[a] ± 0.2	5.5[c] ± 0.3	ND
α-Pinene	14	4.6[a] ± 1.1	ND	43.3[a] ± 53.1	6.2a	55.9[a] ± 71.9	8.2[a] ± 0.2	3.6[a] ± 0.2	ND
Ethyl hexanoate	15	7.2[a] ± 1.4	166.2[a] ± 152.0	99.8[a] ± 12.6	99.6[a] ± 7.4	72.3[a] ± 6.5	127.7[a] ± 97.8	18.9[a] ± 1.6	36.6[a] ± 2.9
Hexyl acetate	16	ND	ND	7.0[b] ± 0.3	11.4[a] ± 0.7	ND	ND	ND	ND
1,8-Cineole	17	7.0[b] ± 1.1	ND	9.5[b] ± 0.3	17.3[a] ± 0.8	7.1[b] ± 0.3	16.2[a] ± 2.5	7.5[b] ± 0.6	ND
D-Limonene	18	2.6[a] ± 0.4	ND	72.7[a] ± 97.6	4.6[a] ± 0.0	41.0[a] ± 53.5	ND	3.5[a] ± 0.5	ND
4-Methoxy-2,5-dimethyl-furan-3-one	19	5.0[c] ± 0.4	27.4[ab] ± 16.4	ND	ND	34.8[a] ± 0.5	22.1[abc] ± 13.0	9.4[c] ± 0.4	7.2[c] ± 0.6
Methyl benzoate	20	87.6[b] ± 17.4	123.5[ab] ± 20.8	5.5[c] ± 0.3	ND	158.5[a] ± 13.6	99.9[b] ± 34.0	28.4[c] ± 1.2	11.3[c] ± 0.9
Methyl octanoate	21	5.9[c] ± 0.6	8.5[a]	6.7[b] ± 0.0	ND	ND	ND	5.1[c] ± 0.1	ND
Ethyl benzoate	22	ND	98.8[a] ± 32.3	ND	ND	58.1[ab] ± 5.6	87.2[a] ± 49.9	6.4[b] ± 0.5	11.6[b] ± 1.5
Ethyl octanoate	B	ND	ND	ND	10.1 ± 1.0	ND	ND	ND	ND

ID, Number or letter used to identify the compound in this work.

ND, not detected.

[a] The quantitative date obtained by calibration curves based on peak area ratio of volatile compounds (pure standards) and docosane as an internal standard.

[b] Values represent mean ± standard deviation of the two replicates.

[c] In each row, the same letters indicate no significant difference by Duncan's multiple range test ($P < 0.05$).

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Table 2. Odor threshold and odor description of volatile compounds identified by GC–MS

Volatile compound	ID	Odor threshold[a] ($\mu g\,kg^{-1}$)	Odor descriptors	KI[e]	Identification[f]
Methyl butanoate	A	60[27,28]	Ether, fruit, sweet[b], caramel[c]	701	MS + KI
Ethyl 2-methyl propanoate	1	7.0[27]	Sweet, rubber[b]	723	MS + KI
Methyl 2-methyl butanoate	2	0.25[27]	Apple[b]	737	MS + KI
Ethyl butanoate	3	1.0[27,28]	Apple[b], sweet, fruity, ester[c]	757	MS + KI
n-Butyl acetate	4	66[28]	Pear[b]	767	MS + KI
Methyl pentanoate	5	20[27]	Sweet, ethereal, apple[d]	777	MS + KI
Ethyl (2*E*)-but-2-enoate	6	Unknown	Sweet, ripe fruit, fruity[c]	795	MS + KI
Propan-2-yl butanoate	7	Unknown	Pungent, fruit[b]	800	MS + KI
Ethyl 2-methyl butanoate	8	0.091[18]	Apple[b], sweet, floral, fruity[c]	812	MS + KI
Ethyl 3-methyl butanoate	9	0.1[29]	Fruity[b], sweet, ripe fruit[c]	814	MS + KI
3-Methylbutyl acetate	10	2.0[27]	Banana[b], plastic, unpleasant, solvent, green[c]	840	MS + KI
Ethyl pentanoate	11	1.5[27,28]	Yeast, fruit[b], green, herbal, grassy, floral[c]	872	MS + KI
Methyl hexanoate	12	70[27,28]	Fruit, fresh, sweet[b], eucalyptus[c]	906	MS + KI
Benzaldehyde	13	350[30]	Almond, burnt sugar[b], plastic, green, fruity[c]	922	MS + KI
α-Pinene	14	62[18]	Pine, turpentine[b]	927	MS + KI
Ethyl hexanoate	15	0.01[18]	Apple peel, fruit[b], sweet, minty[c]	980	MS + KI
Hexyl acetate	16	10[18]	Fruit, herb[b]	993	MS + KI
1,8-Cineole	17	12[30]	Mint, sweet[b]	1014	MS + KI
D-Limonene	18	10[18]	Citrus, mint[b]	1018	MS + KI
4-Methoxy-2,5-dimethyl-furan-3-one	19	0.03[30]	Caramel, sweet, mildew[b]	1025	MS + KI
Methyl benzoate	20	0.52[29]	Prune, lettuce, herb, sweet[b]	1063	MS + KI
Methyl octanoate	21	200[27]	Orange[b]	1104	MS + KI
Ethyl benzoate	22	60[30]	Camomile, flower, celery, fruit[b]	1139	MS + KI
Ethyl octanoate	B	5[18]	Fruit, fat[b], coconut, floral[c]	1179	MS + KI

ID, Number or letter used to identify the compound in this work.
[a] Odor threshold in water from Refs 18,27–30.
[b] From Ref. 12.
[c] From Ref. 11.
[d] From The Pherobase (http://www.pherobase.net)
[e] Kovats indices (KIs) were calculated in relation to the C_7–C_{26} *n*-alkanes for a HP-Ultra 1.
[f] Mass spectra and Kovats indices (MS + KI) of each volatile compound detected in murtilla fruit aroma agree with MS and KI of the correspondent pure standard (Sigma Aldrich).

Table 3. Odor activity values of volatile compounds considered as potentially aroma-contributing substances to murtilla fruit aroma

Odor activity value (OAV) range[b]	Ecotype 14-4[a]		Ecotype 17-2[a]		Ecotype 19-1[a]		Ecotype 33-5[a]	
	Initial	End	Initial	End	Initial	End	Initial	End
OAV > 1000		15 (16 628)	15 (9978)	15 (9961)	15 (7228) 19 (1162)	15 (12 767)	15 (1891)	15 (3662)
999 > OAV > 100	15 (718) 2 (493) 19 (168) 20 (168)	8 (982) 19 (913) 20 (237) 3 (154)	8 (879) 2 (509) 9 (385) 3 (162)	8 (374) 9 (127)	8 (549) 20 (305) 2 (293) 3 (143) 9 (130)	8 (787) 19 (735) 20 (192) 3 (121)	2 (322) 19 (314) 8 (129)	19 (240) 8 (151)
99 > OAV > 10	8 (45) 3 (21)	2 (95)	20 (11)	3 (91) 2 (63)		2 (34)	20 (55) 9 (40)	3 (43) 20 (22) 2 (21)

[a] Volatile compounds: 2 = methyl 2-methyl butanoate; 3 = ethyl butanoate; 8 = ethyl 2-methyl butanoate; 9 = ethyl 3-methyl butanoate; 15 = ethyl hexanoate; 19 = 4-methoxy-2,5-dimethyl-furan-3-one; 20 = methyl benzoate.
[b] Odor activity values showed in parenthesis were estimated dividing the concentration of compounds ($\mu g\,kg^{-1}$, Table 1) by their published odor threshold showed in Table 2.

values (OAVs) estimate by ratio of volatile compound concentration to odor threshold (Table 2) publishing in the literature.[18,27–30]

The odor activity value (OAV) was estimated for analyzing the potential importance of the volatile compounds for aroma released by the four ecotypes of murtilla fruit at the initial and end of the storage. Odor activity values (OAVs; Table 3) were calculated dividing the concentration of compounds ($\mu g\,kg^{-1}$, Table 1) by their published odor threshold[18,27–30] shown in Table 2.

Usually, volatile compounds with concentrations higher than their odor threshold (OAV > 1) are mainly considered as aroma-contributing substances.

However we considered volatile compounds with OAVs higher than 10 following the suggestion reported by Qian and Wang.[18]

According to the OAVs (Table 3), the most important odorant of murtilla fruit were ethyl hexanoate (15; apple peel, fruit, sweet and minty notes) and 4-methoxy-2,5-dimethyl-furan-3-one (19; caramel and sweet notes) because they showed the more high OAVs. Ethyl hexanoate has been considered as a potent odorant compound in some varieties of blackberries because is one of the constituent with the highest odor activity values.[18] The lowest odor threshold and the low concentrations showed by 4-methoxy-2,5-dimethyl-furan-3-one (mesifurane) (5–34.8 $\mu g\,kg^{-1}$ fresh weight) in ecotypes 14-4, 19-1 and 33-1 resulted in high OAVs, signaling this odorant as another key compound to murtilla fruit aroma. Mesifurane is an analog compound of the furaneol (4-hydroxy-2,5-dimethyl-furan-3-one) that has been reported as the most important component for strawberry aroma. The furaneol concentration in three strawberry varieties fluctuated throughout the ripening process, reaching the maximum value (2.96 $mg\,kg^{-1}$) for the Carezza variety and the minimum value (0.42 $mg\,kg^{-1}$) for the Darselect variety.[16]

Volatile compounds with OAVs between 100 and 999 (Table 3), such as methyl 2-methyl butanoate (2), ethyl butanoate (3), ethyl 2-methyl butanoate (8), ethyl 3-methyl butanoate (9) and methyl benzoate (20) also are potentially active odorant to aroma of murtilla fruit. Ethyl butanoate, which has been described as a fruity odor showed the highest intensity in the aroma of unpasteurized orange juice as measured by the mean of the Osme gas chromatography–olfactometry technique. The aroma of excessively heated orange juice also showed a strong intensity of ethyl butanoate.[31] Ethyl 2-methyl butanoate has been reported like a volatile with high odor activity values (OAV > 10) in blackberries.[18] Recently Pino and Mesa,[29] using values based on OAVs, reported that 4-methoxy-2,5-dimethyl-furan-3-one, ethyl butanoate and methyl benzoate were potentially most important compounds to aroma from 20 cultivars of mango.

Considering the odor descriptor (Table 2) of these seven odorants, the murtilla fruit aroma could be characterized as a mixture of fruity, sweet and floral notes.

Effect of the storage on the volatile compounds

Twenty-one volatile compounds identified at the initial of the storage were also present after 60 days in all the ecotypes evaluated at 0 °C (Table 1). Propan-2-yl butanoate (7) was not present in the aroma of any murtilla fruit ecotype at the end of the storage time. This fact could be due to modifications in the metabolic routes of the volatile compounds formation that occur during fruit ripening and may be influenced by the storage conditions.[32] Two new volatile components (A and B, Table 1) identified at the end of the storage period are also found in the aroma of the strawberry, blackberry and some tropical fruits. Methyl butanoate, present in low concentrations in the murtilla aroma, has been considered to be an important aroma in the fresh strawberry aroma quality.[16] This compound has also been reported in the aroma composition of the tropical fruits acerola, cupuaçu, soursop and cashew apple.[23] Ethyl octanoate was identified in low concentration in blackberry volatile composition, in several tropical fruits aroma and in juices and nectars from the traditional fruits.[18,23,25]

There were both qualitative and quantitative modifications in the volatile composition of the murtilla fruit aroma found between the initial and end of the storage period for all ecotypes. This finding indicates that the experimental temperature was not able to retard the murtilla fruit modifications that affect the metabolic routes related with the formation of the aroma volatile components. The temperature in our experiment (0 °C) was selected by Ayala-Zavala *et al.*[17] as the best to maintain the overall quality of strawberry storage during 14 days at 0, 5 and 10 °C. Nevertheless, these researchers reported that the storage temperature at 10 °C positively enhanced the production of aroma compounds from strawberry and no significant differences in the peak area replicates of the evaluated volatile compounds were detected during the storage.

Our result indicates that it could be necessary to control parameters other than temperature, such as humidity, CO_2 and ethylene, during cooled storage of the murtilla fruit to maintain an uniform volatile composition.

Methyl hexanoate showed the highest concentration at the beginning of the storage in three ecotypes (Table 1). All ecotypes showed a decrease in the concentration of the methyl hexanoate after 60 days at 0 °C, but only for the 17-2 ecotype was the difference significant ($P < 0.05$) between the initial and the end storage period. This result is supported by Ayala-Zavala *et al.*,[17] who reported a decrease in methyl hexanoate in the aroma of the strawberry at the end of the storage period at 0, 5 and 10 °C. On the contrary, Azodanlou *et al.*[16] reported an increase in the concentration of this compound during the ripening process of three strawberry varieties. These results suggest a higher amount of methyl hexanoate at the initial stage of the storage in three ecotypes followed by the respective decrease, indicating that these murtilla fruit were harvested when they were ripe. On the contrary, ethyl hexanoate showed an increasing trend at the end of the storage for ecotypes 14-4, 19-1 and 33-5, but maintained constant in the ecotype 17-2.

Methyl benzoate was present in a high concentration in the aroma from ecotype 19-1 and showed a statistical decrease ($P < 0.05$) at the end of the storage period. The amount of methyl benzoate at the beginning and the end of the experiment were not significant ($P > 0.05$) in ecotypes 14-4 and 33-5.

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Similar results were obtained when ethyl benzoate concentration in the aroma of the ecotypes 19-1 and 33-5 was evaluated. The highest amount of ethyl benzoate was determined for ecotype 14-4 at the end of the storage period and was statistically different ($P < 0.05$) with the concentration presented by ecotype 33-5 (Table 1).

The concentration of methyl 2-methyl butanoate decreased significantly at the end of the storage period ($P < 0.05$) in all the ecotypes. The amounts of ethyl butanoate and ethyl 2-methyl butanoate increased significantly at the end of the experiment for ecotype 14-4. Nevertheless, the variation in the amounts of these compounds for the others three ecotypes were not significant ($P > 0.05$). The decreasing in concentration of ethyl 3-methyl butanoate was significantly ($P < 0.05$) for ecotype 17-2.

As mentioned previously, it is not uncommon to find in the literature qualitative and quantitative differences in volatile composition between varieties or cultivars fruits. For instance, different strawberry varieties (Carezza, Darselect and Marmolada) have shown significant differences ($P < 0.05$) in the concentration of several volatile compounds.[16] In blackberries, the 'Thornless Evergreen' cultivar presented more volatiles than 'Marion' variety.[18] Guillot *et al*.[26] reported differences in the concentrations by means of gas chromatography–olfatometry analysis of 10 volatiles compounds, previously identified as key aroma compounds in six apricot varieties.

In general, the statistical analysis showed a strong effect of the storage on the concentration of volatile compounds released from all fruit ecotypes evaluated, suggesting that it could have differences associated with intrinsic characteristic of these ecotypes. From the seven (2, 3, 8, 9, 15, 19 and 20 in Table 3) volatile compounds that are potentially important to aroma characteristic of murtilla fruits, the concentration of methyl 2-methyl butanoate (2) was the more affected by the cooling storage and, consequently, the OAV was affected.

Based on the seven potentially important odorants, it is possible to consider that the aroma release by the 19-1 murtilla fruit ecotype was the most stable during the storage period because the OAVs were always higher than 100, except for methyl 2-methyl butanoate at the end of the period.

CONCLUSIONS

The volatile compounds identified in murtilla fruit aroma have been found in other aromatic fruits (tropical and traditional) widely consumed around the world. The major concentration of volatile compounds in murtilla fruit aroma produced by all the ecotypes were methyl 2-methyl butanoate, ethyl butanoate, ethyl 2-methyl butanoate, methyl hexanoate, ethyl hexanoate, methyl benzoate and ethyl benzoate.

Based on estimated odor activity values, ethyl hexanoate and 4-methoxy-2,5-dimethyl-furan-3-one were classified as the most potent compounds to murtilla fruit aroma. Methyl 2-methyl butanoate, ethyl butanoate, ethyl 2-methyl butanoate, ethyl 3-methyl butanoate and methyl benzoate are potentially important aroma compounds because showed OAVs over 10.

The murtilla fruit aroma characteristic may be described as a mixture of fruity, sweet and floral notes.

The statistical analysis showed that the storage for 60 days at 0 °C produced a distinct effect on the volatile compounds released from the all murtilla fruit ecotypes, suggesting that it could have differences associated to own nature of these ecotypes. The murtilla fruit from ecotype 19-1 could be considered the best for cooling storage because OAVs of the seven potentially most potent odor compounds were over than 100 during this period, excepting for methyl 2-methyl butanoate at the end of the storage.

Future studies to evaluate the effect of the cooling storage on the aroma of the murtilla fruit will need to be carried out to consider different packaging and temperature and others parameters such as humidity and CO_2 for more effective controlling in the released of the volatile compounds.

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