Determination of Volatile Flavor Components, Sugars, and Ascorbic, Dehydroascorbic, and Other Organic Acids in Calamondin (*Citrus mitis* Blanco)

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The volatile flavor components of calamondin juice were determined by a headspace analysis technique. Twenty components including five aldehydes (acetaldehyde, decanal, nonanal, octanal, and perillaldehyde), two esters (geranyl acetate and neryl acetate), five alcohols (ethanol, linalool, methanol, terpinen-4-ol, and α-terpineol), and eight hydrocarbons (3-carene, limonene, myrcene, α-pinene, β-pinene, τ-terpinene, terpinolene, and valencene) were identified. The levels of these components, except for limonene and myrcene, were determined in the juice. The levels of individual sugars (glucose, fructose, and sucrose) and dehydroascorbic, ascorbic, citric, and malic acids were also given in this study.

INTRODUCTION

Calamondin, or calamansi, is the leading citrus fruit in the Philippines that is used primarily for its juice (Nisperos et al., 1982). It is considered as a substitute for lemon. The fruit, believed to be a hybrid of *Citrus reticulata* Blanco × *Fortunella* spp., has been distributed throughout tropical (Philippines and Central America) and subtropical (Japan and Florida) areas (Mabesa, 1990; Morton, 1987; Hodgson, 1967). Although it is produced commercially in other countries, the potential for expanded production of the fruit in the United States does not appear to be great because of the availability of other citrus fruit with identical uses (Crane and Campbell, 1990). Sufficient information is available on postharvest physiology, handling, and processing of the fruit, as well as the nutritional and chemical changes during processing and storage of the juice (Mabesa, 1990; Nisperos et al., 1982; Mendoza and Pantastico, 1979). However, studies on the volatile flavor constituents are limited. So far, only six flavor components have been identified in the juice (Mina, 1980).

This study was therefore conducted to provide qualitative and quantitative data on the different volatile components, as well as quantitative data on sugars and ascorbic, dehydroascorbic, and other organic acids of calamondin.

MATERIALS AND METHODS

Mature calamondin fruits (100–120), obtained from the Citrus Arboretum, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Winter Haven, FL, were extracted by cutting the stem end and squeezing manually since the fruit were small (about 2–2.5 cm in diameter). The juice was strained through a 12-mesh stainless steel sieve to remove the seeds to provide the calamondin juice used in the study.

The volatile flavor components were analyzed by transferring six 2-mL samples of juice into six 10-mL vials equipped with crimp-top caps with TFE/silicone septa seals. The different components were determined by headspace analysis on a Perkin-Elmer Model 8500 gas chromatograph equipped with an HS-6 headspace sampler, a 0.53 mm × 30 m polar Durowax column, and an FID detector, as described in an earlier study (Nisperos-Carriedo and Shaw, 1990). Juice samples were equilibrated in the headspace sampler for 15 min at 80 °C prior to injection. Injection parameters for the headspace sampler were 0.5-min vial pressurization time followed by 0.02-min injection time.

Column oven temperature programming was 40 °C for 6 min and then raised at 6 °C/min to 180 °C. The different components were identified by comparison of retention times with those of standards and by enrichment of juice with authentic samples. Concentrations were calculated by comparing peak heights with those of standards, determined by making five injections of known concentrations of each component added to a juice base to obtain average peak height. The standard concentrations (parts per million) were the following: acetaldehyde, 2.4; decanal, 1.3; nonanal, 0.5; octanal, 1.3; perillaldehyde, 0.5; geranyl acetate, 2.5; neryl acetate, 0.3; ethanol, 192.4; linalool, 0.9; methanol, 10.0; terpinen-4-ol, 0.4; α-terpineol, 24.0; 3-carene, 0.5; α-pinene, 7.0; β-pinene, 2.0; τ-terpinene, 3.0; terpinolene, 0.4; and valencene, 0.2. The standard solutions were prepared by addition of 5 μL of an aqueous ethanolic solution of standards to 20 mL of bland juice prepared by dilution of concentrated orange juice containing no added volatile components (evaporator pumpout) to 11.9° Brix. A similar juice sample was analyzed by gas chromatography-mass spectrometry (GC-MS). The juice sample (100 mL) was extracted with methylene chloride (99.9% GC purity). The extract was saturated with sodium sulfate to remove water and then filtered and concentrated by distillation to give a sample of anhydrous extract before introduction into the GC-MS. A Hewlett-Packard Model 5970B, MSD, GC-MS was used with a 0.31 × 50 m fused silica column of cross-linked 5% phenylmethyl silicone. The different components were identified by comparing the mass spectra and retention times with those of known compounds.

Ascorbic, dehydroascorbic, and other organic acids were determined by HPLC. Juice (50 mL) was blended with 0.05 N H₃PO₄, pH 2.3 (50 mL) for 3 min. The slurry was centrifuged, and the supernatant was collected and made to 100 mL with the 0.05 N H₃PO₄ solution to give the extract. The extract was purified by passing 3 mL through a disposable C₁₈ Sep-Pak cartridge (Waters Associates), previously preconditioned by flushing with acetonitrile (2 mL) followed by double-distilled water (5 mL), and a 0.45-μm Millipore filter prior to injection. The chromatographic equipment consisted of a solvent delivery system (Perkin-Elmer isocratic LC pump 250) and a 20-μL sample injector. Detection of the acids was performed at 215 and 260 nm using a diode array detector (Perkin-Elmer LC 235) interfaced with two integrators (Hewlett-Packard 3396A) and a chromatography data system (Chrom Perfect 2, Justice Innovations). Although ascorbic acid has a minimum absorbance at 245 nm, detection was performed at 260 nm to avoid some interference in measuring ascorbic acid (Finley and Duang, 1981). Because of absorption by interfering compounds at 215 nm, dehydroascorbic acid was quantified by obtaining the difference in absorbance between 215 and 260 nm (Nisperos-Carriedo et al.,
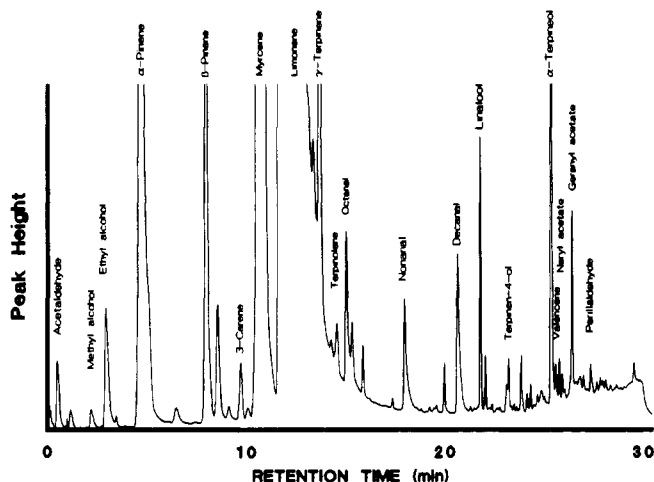
Figure 1. Volatile flavor components of calamondin as determined by headspace analysis using a polar Durowax column (0.53 mm × 30 m, 1.0-μm film thickness).

Table I. Volatile Flavor Components of Calamondin Juice

component	concn,[a] ppm	component	concn,[a] ppm
aldehydes		esters	
acetaldehyde	2.4	geranyl acetate	2.5
decanal	1.1	neryl acetate	0.3
nonanal	0.5	hydrocarbons	
octanal	1.2	3-carene	0.2
perillaldehyde	0.4	limonene	nd[b]
alcohols		myrcene	nd
ethanol	11	α-pinene	5.6
linalool	0.9	β-pinene	2.0
methanol	9	τ-terpinene	3.0
terpinen-4-ol	0.2	terpinolene	0.3
α-terpineol	20.6	valencene	0.2

[a] Values represent means of six determinations. [b] nd, not determined.

1992). A Spheri-5 RP-18 (110 × 4.6 mm) column and two Polypore H columns (110 × 4.6 and 220 × 4.6 mm) purchased from Brownlee Labs, Inc. (Santa Clara, CA) were used for the separation. The columns were equilibrated at room temperature with the mobile phase (2% KH_2PO_4, pH 2.3), after which the standard mixture or sample was introduced into the column. The flow rate used was 0.4 mL/min with an accompanying pressure of 670–700 psi.

Sugar analyses were accomplished according to the procedure described by Baldwin et al. (1991).

RESULTS AND DISCUSSION

The unique flavor of calamondin, just as with other citrus cultivars, can be attributed to the presence of the different volatile components, including those presented in Figure 1 and Table I. Some constituents such as acetaldehyde, ethanol, limonene, linalool, terpinen-4-ol, and α-terpineol have been identified in calamondin before (Mina, 1980), but the rest of the components are identified and quantified in calamondin for the first time. Calamondin juice contains volatiles that have been considered important to the flavor of other citrus varieties, such as mandarin, lemon, lime, kumquat, and orange flavor (Shaw, 1991). Belingheri et al. (1991) have reported that β-selinene and limonene, together with small amounts of oxygenated terpenes, are responsible for the fragrance of calamondin fruit. In this study, however, β-selinene was not detected at all. This compound may only be present in the epicarp, or its level in the juice may be too low to be detected. The relative abundance of limonene can be noticed (Figure 1), although the level was not determined. Limonene was not soluble in the standard aqueous ethanolic mixture used for GC calibration at the relatively high level needed,

Table II. Sugars and Ascorbic, Dehydroascorbic, Citric, and Malic Acid Contents of Calamondin Juice

compound	concn[a]	compound	concn[a]
sucrose	1.1%	ascorbic acid	44.5 mg/100 g
glucose	1.2%	dehydroascorbic acid	2.2 mg/100 g
fructose	1.3%	citric acid	3.6%
		malic acid	0.2%

[a] Values represent means of five determinations.

and so its level has to be determined by a separate method, the Scott oil determination method (Scott and Veldhuis, 1966). However, due to the limited amount of sample, this compound was not quantified. The presence of another hydrocarbon, myrcene, was also detected in calamondin juice. Its level, however, cannot be determined due to its insolubility in the ethanol mixture of standards. The component perillaldehyde has been identified in tangerine, lemon, and lime essences in previous studies (Shaw, 1977; Moshonas and Shaw, 1972a,b). All of the components reported here may be contributory to calamondin flavor; however, the relative contribution of each of these components to calamondin flavor has not been determined.

Table II presents the values for individual sugars and ascorbic, dehydroascorbic, citric, and malic acids. The fructose and glucose levels are within the range given for oranges and tangerine (1–2.5%), while the sucrose level is lower than the range for oranges and tangerines (2–5%) and higher than that reported for lemons and limes (0.2–0.3%) (McCready, 1977). Values obtained for ascorbic and dehydroascorbic acid were within the range reported for fresh citrus fruit, 29.1–63.4 and 0.3–9 mg/100 g, respectively (Wills et al., 1984; Wimalasiri and Wills, 1983; Kefford and Chandler, 1970). Calamondin juice is characterized by its high acidity, as reflected by the high citric acid content. The obtained value for citric is slightly lower than that of lemon (4.0–5.0%), while malic acid is within the ranges given for tangerine, lemon, and lime (0.06–0.26) (Yamaki, 1989; Clements, 1964).

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Registry No. Acetaldehyde, 75-07-0; decanal, 112-31-2; nonanal, 124-19-6; octanal, 124-13-0; perillaldehyde, 2111-75-3; geranyl acetate, 105-87-3; neryl acetate, 141-12-8; ethanol, 64-17-5; linalool, 78-70-6; methanol, 67-56-1; terpinen-4-ol, 562-74-3; α-terpineol, 98-55-5; 3-carene, 13466-78-9; limonene, 138-86-3; myrcene, 123-35-3; α-pinene, 80-56-8; β-pinene, 127-91-3; τ-terpinene, 99-85-4; terpinolene, 586-62-9; valencene, 4630-07-3; sucrose, 57-50-1; glucose, 50-99-7; fructose, 57-48-7; ascorbic acid, 50-81-7; dehydroascorbic acid, 490-83-5; citric acid, 77-92-9; malic acid, 6915-15-7.