Citrus materials

Composition of Myrtle Leaf Orange

An in-depth analysis of endocarp composition of this interesting traditional Italian/Sicilian/Calabrian material

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Myrtle leaf orange is known as *chinotto* in Italy and *chinois* in France, presumably because the fruit originated in China. The myrtle leaf orange is considered a botanical variety of the bitter orange (*Citrus aurantium* var. *myrtifolia*), but there are some who believe it either should **a)** be promoted to the rank of species (*Citrus myrtifolia* Raf., Tanaka Classification), or **b)** be equated to the *Citrus aurantium* L. as a subspecies.

Plant: The plant is farmed in Italy, particularly in Liguria, near Savona, between Finale Ligure and Varazze, with an annual production of 20-30 quintals (about 4,400-6,600 lbs); small orchards also are present in Sicily and Calabria. The plant has the appearance of a small bush with thornless branches loaded with small dark green elliptical leaves. The bush is characterized by limited growth of between 1-2 m. The fruit, picked between December and March, is spherical but rather flat at the base; it has a very thin, fragrant skin of a color similar to that of the mandarin. Its endocarp, divided into eight to 10 segments, contains small seeds and a small quantity of bitter acidic juice.

The fruits, which may remain on the tree for up to two years, are the same size as apricots and are classified commercially as:

- regular: from 18-50 g,
- medium: from 9-17 g,
- small: < 9 g

Varying applications: The small fruits — usually about 3 cm in diameter — are picked unripe in December and used in the production of so-called "chocolate Boers" (liqueur-filled chocolates with a small candied fruit inside). The larger ripe fruits are picked in February and March; they are too bitter and acidic to be used fresh, although their juice can be applied in jams, prepared as candied fruit or added to syrup. The candying process, which transforms the fruit into a bittersweet delicacy, involves a process called "turning," in which the thin epicarp is removed and the fruit is boiled in syrups with increasing concentrations of sugar.

Because of its aroma and thickness, the peel is well suited to the preparation of fizzy drinks, bitter digestive liqueurs and nonalcoholic aperitifs (bitters). Considering the growing interest in upgrading of products with materials tied to the traditions and cultures of particular areas — and the fact that there is data available in the literature referring to the

Examination at a glance …

The authors studied the composition of the endocarp of myrtle leaf orange fruits. Their analyses regarded the following: optical residue, expressed in °Brix; total acidity (g/kg), expressed as monohydrate citric acid; formol number (mL of NaOH 0.1 N per 100 g of endocarp); ascorbic acid (mg/kg); organic acids (citric, isocitric and malic) (g/kg); sugars (sucrose, glucose, fructose) (g/kg); mineral components: sodium, potassium, calcium, magnesium and phosphorus (mg/kg); ashes (g/kg); and total flavonoids (mg/ kg). And, finally, a specific study was made of the flavanone glycosides and the free amino acids.

essential oil of myrtle leaf orange and its juice — it appears useful to study the composition of the endocarp of the fruit.[1-3]

Materials and Methods

The myrtle leaf oranges from Calabria and Sicily that were picked in December (Calabria) and March (Sicily) were green and orange, respectively, weighing between 30-50 g. The endocarp was obtained from peeled fruits, cut into small pieces, separated from the seeds, crushed in a mixer and puréed in a sifter equipped with a sieve with 0.45-mm holes.

The purée obtained then was used to carry out the following analytical determinations using the methodology contained in the R.S.K. (Richtwert Schwankungsbrüte Kenzahl) standards[4]: the optical residue was expressed in °Brix; total acidity (g/kg) was expressed as monohydrate citric acid; formol number (mL of NaOH 0.1 N per 100 g of endocarp); ascorbic acid (mg/kg); organic acids (citric, isocitric and malic) (g/kg); sugars (sucrose, glucose and fructose) (g/kg); mineral components: sodium, potassium, calcium, magnesium and phosphorus (mg/kg); ashes (g/kg); total flavonoids (mg/ kg); the specific analysis of the flavanone glycosides and free amino acids was carried out via HPLC.

Flavanone glycosides: The flavanone glycosides were extracted from the purée with DMF (N,N'-dimethylformamide) and analyzed by HPLC, using coumarin as the internal standard. Standard solutions of the various flavonoids were obtained by solubilizing 100 mg of flavanone glycosides in 100 mL of a water-DMF mixture (1:1, v/v) from which solutions with a concentration of 50 mg/l were prepared, with appropriate dilutions.[5]

Amino acids: The quantitation of free amino acids was carried out by high-pressure liquid chromatography (HPLC), using α-aminobutirric acid (0.5 mM) as the internal standard. A quantity of purée (about 15 g) was centrifuged at 10.000 revs/min for 10 min; 5 mL of serum was loaded onto a Bio-RAD resin column (AG in 50 W-X 8H$^+$) connected to a water vacuum pump; after washing with 50 mL of water (HPLC grade), which was discarded, the amino acids were eluted with 25 mL of an aqueous solution of NH_4OH, diluted with water (1:1, v/v). The eluate was collected in a 250 mL Rotavapor flask and dried in a vacuum at 55°C. The residue was recovered with 10 mL of HCl 1N mixed, at a ratio of 1:1, with α-aminobutirric acid (0.5 mM) (internal standard).

The sample thus obtained (20 µL) was derivatized with phenylisothiocyanate (PITC) so as to obtain the phenylthiocarbamilderivates (PTC), which can be detected with UV at 254 nm.[6]

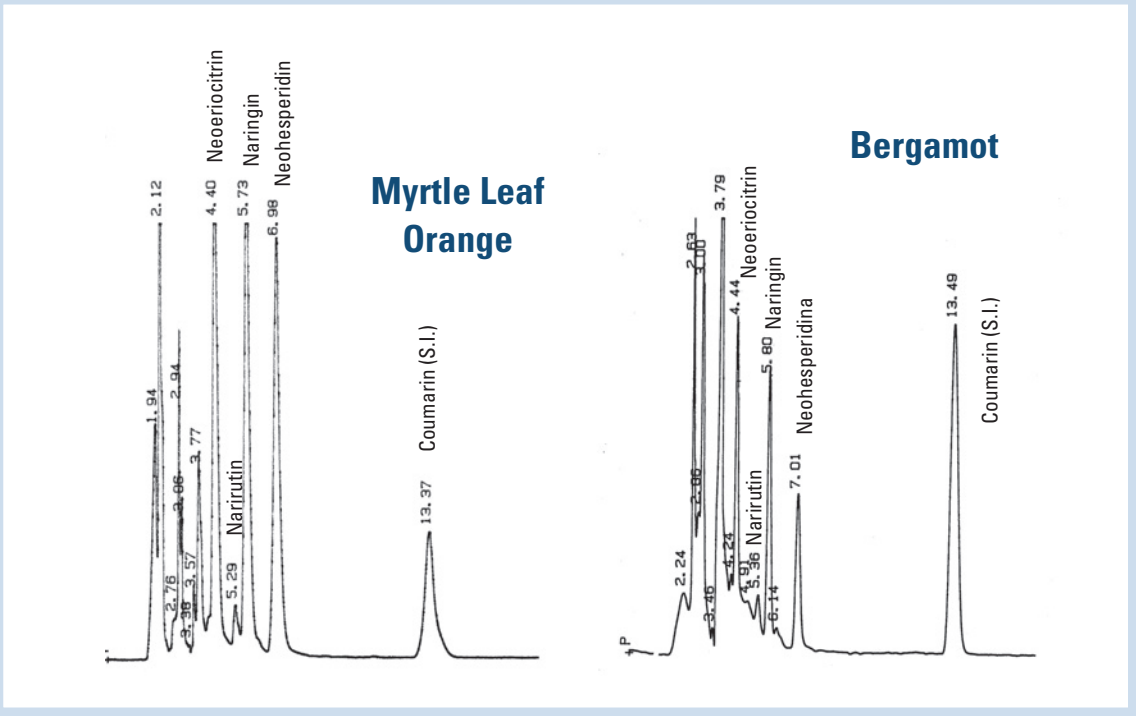
The PTC solution was dried in a vacuum (65 mTorr); the residue was recovered with 200 µL of phosphate buffer (Na_2HPO_4) at pH 7.4 and at 50 percent (v/v) of acetonitrile and analyzed by HPLC, according to the method suggested by Bidlingmeyer and Coll.[7]

Principal characteristics of the composition of the endocarp of myrtle leaf orange **T-1**

Month of picking		December	March
optical residue	(g/100 g)	7.80	11.20
total acidity	(g/kg)	6.4	6.6
anhydrous citric acid	(g/kg)	2.7	4.3
L-malic acid	(g/kg)	3.0	1.2
D-isocitric acid	(mg/kg)	n/a	n/a
L-ascorbic acid	(mg/kg)	22	124
formol number (mL NaOH 0.1 N/100 g of endocarp)		41	25
glucose	(g/kg)	14	24
fructose	(g/kg)	18	26
sucrose	(g/kg)	5	14
total sugars	(g/kg)	37	64
ashes	(g/kg)	5.8	5.5
sodium	(mg/kg)	21	22
potassium	(mg/kg)	2,670	2,010
calcium	(mg/kg)	115	169
magnesium	(mg/kg)	104	92
phosphorus	(mg/kg)	323	352

Flavonoid profiles of myrtle leaf orange endocarp and bergamot juice; operating conditions: chromatograph: HPLC HP 1090; column: ODS C_{18}, mM 200 x 4,6 i.d.,10 μm; eluent: water: acetonitrile, 75:25; flow: 0.7 mL/min; detector: U.V. 287 nm wavelength; volume injected: 20 μL; internal standard: coumarin

F-1



Results and Discussion

The main analytical characteristics of the myrtle leaf orange endocarp are shown in T-1.

The most representative carbohydrates were glucose and fructose, while sucrose was present in lower concentrations. The glucose/fructose ratio was 0.78 and 0.92, respectively, for December and March myrtle leaf oranges.

Among the organic acids, those present in greater quantities were citric and malic. D-isocitric acid could not be dosed; a considerable difference could be seen between the ascorbic acid contents of December and March myrtle leaf oranges. Moreover, it should be noted that the ash and mineral element contents were very high. The quantities of sodium and potassium were similar to those contained in other citrus fruits. Potassium levels varied between 2,010- 2,670 mg/kg. Concentrations of calcium and magnesium were high, with values varying between 115-169 mg/kg and between 92-104 mg/kg, respectively. The formol number, linked to the amino acid content, varied between 24-40.

The total flavonoid content (expressed as naringin) of myrtle leaf oranges picked in December and March proved to be 3,700 mg/kg and 2,900 mg/kg, respectively. As can be seen from the chromatographic profile HPLC (F-1), the main flavanone glycosides were naringin, neohesperidin and neoeriocitrin, along with small quantities of narirutin and traces of hesperidin.

Glucosidal flavonoid composition (mg/kg) of the endocarp of myrtle leaf orange **T-2**

Month of picking	December	March
total flavonoids*	3,700	2,900
neoeriocitrin	700	560
narirutin	65	60
naringin	1,600	1,300
neohesperidin	1,100	800

*by Davis test determined

The concentrations of these flavonoids, shown in T-2, demonstrate that naringin was the most plentiful flavonoid. Its concentrations varied between 1,300-1,500 mg/kg, followed by neohesperidin, with values between 800-1,500 mg/kg, and neoeriocitrin, with values between 560-700 mg/kg. The concentrations of narirutin were much lower, with values between 60-65 mg/kg.

Regarding the free amino acid composition, the data in T-3 shows the presence of 16 amino acids. Proline, asparagine, aspartic acid, arginine, GABA (γ- aminobutirric acid) and serine are those present in the greatest quantities, representing 80 percent of the total amino acids. Other amino acids present in appreciable quantities are glutamic acid, threonine, leucine and phenylalanine. Finally, histidine, tyrosine, ornithine, valine, isoleucine and glycine are present in small quantities and make up 1.36 percent of the total amino acid content.

Free amino acid composition (mg/kg) of the endocarp of myrtle leaf orange **T-3**

Month of picking	December	March
aspartic acid	273.04	391.02
glutamic acid	85.50	194.80
serine	324.20	299.18
asparagine	2,661.60	785.04
glycine	n.r.	37.60
histidine	23.28	8.18
GABA	385.62	340.98
threonine	67.90	55.66
arginine	729.90	354.50
proline	1,102.60	1,184.38
tyrosine	21.30	15.62
valine	29.20	22.30
isoleucine	19.76	12.80
leucine	51.52	38.42
phenylalanine	64.12	47.68
ornithine	39.40	21.34
	5,878.94	3,809.50

Conclusion

Comparing the chromatograms (HPLC) of the flavonoids of myrtle leaf orange and bergamot (F-1) from a qualitative point of view, it can be seen that the same flavonoids are present in both: naringin, neohesperidin, neoeriocitrin and narirutin. Hesperidin was present in traces that could not be dosed; at the same time, the concentrations of the individual flavonoids in the myrtle leaf orange purée were much higher than in the bergamot juice.[8] Regarding the amino acid composition, in accordance with the results obtained in myrtle leaf orange juice by Calvarano, those present in greater quantities were proline, asparagine and aspartic acid.[3]

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