

Chemical Composition of Chinotto Juice [†]

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Abstract: *Citrus x myrtifolia* (Rafinesque) fruits are commonly used to produce the popular Italian beverage ‘Chinotto’. The *C. myrtifolia* plant comes from Asia, as most of *Citrus* spp., but nowadays is spread in Mediterranean countries and in Italy, mostly Liguria and Sicily. The fresh juice obtained by squeezing ripe fruits of Chinotto has been investigated with the aim to draw up guidelines to be used as a marker of quality and authenticity of this product. The juice composition was studied in terms of soluble solids, organic acids, titratable acidity, sugars, mineral components, flavanone glucoside and ascorbic acid content. The results represent a starting point to define the quality of chinotto juice, improving its quality and detecting any adulterations or frauds.

Keywords: citrus myrtifolia; chinotto; flavonoids; chemical composition

1. Introduction

Citrus x myrtifolia Raf., commonly known as chinotto or myrtle-leaved orange, is a plant species belonging to the Rutaceae family, subfamily Aurantioideae and genus *Citrus*, which originates from a mutation of sour orange *C. aurantium* var. *myrtifolia* [1]. Native of southern China, its origin has not been exactly ascertained. The plant was cultivated for centuries in France and Italy. In Italy the production of chinotto fruits are concentrated in the southern regions, Sicily and Calabria, and in the Ligurian coast.

The unripe fruits look like small green aromatic tangerines, while mature fruits are bigger and orange painted. The flesh is bitter and sour and divided into 8–10 segments [2].

Although in many countries it is grown only for ornamental purposes, its sour-tasting fruits have a significant impact on the food industry, indeed the juice and the fruits extract are an essential flavor component of syrups, soft drinks and aperitifs and, above all, the primary ingredient of the ‘Chinotto’ Italian soft drink.

The AIJN, Association of the Industry of Juices and Nectars of the European Union (AIJN), has established reference guidelines for fruit juices [3], but there is no information for chinotto. The aim of this work has been help to fill the knowledge gaps in authentic composition for Chinotto juice, obtained from the edible part of fruits by mechanical processes. Hence, it will possible establish some reference guidelines for this product that protect consumers from food fraud ensuring authenticity.

2. Experiments

2.1. Reagents and Standards

All reagents and solvents were of analytical grade and were purchased from Sigma Aldrich S.r.l. (Milan, Italy). Enzymatic kit for sugars (glucose, fructose and saccharose) organic acids (D-isocitric, and D-L lactic acid) and ethanol determinations were obtained from RBiopharm (Darmstadt, Germany).

2.2. Plant Materials

In this study 10 fruit batches (about 3 Kg of product for each batch) were harvested in the period from December 2018 to March 2019 at SSEA arboretum (Reggio Calabria, Italy) and placed in a 4 °C refrigerated box to be processed.

All samples were thoroughly washed to remove metal residues and pollutants from the exocarp; the juice was then extracted using a manual citrus squeezer and subsequently filtered with a 1.18 mm diameter steel mesh filter. The juices were then packaged in 50 mL plastic containers and immediately stored at -20 °C until analysis.

2.3. Analytical Reference Methods

The determination of physicochemical parameters, of Chinotto juices were quantified by applying the IFU (International Federation of Fruit Juice Producers) reference methods [4].

The Soluble solids (TSS), expressed in °Brix, were determined by means of measuring refractive index at 20 °C using a digital refractometer (Mettler-Toledo S.p.A; Milan Italy) according to IFU method n. 8. Relative density at 20°C was determined with IFU method n. 1. Titratable acidity was measured according to the IFU method n. 3. Formol number, expressed as mL of NaOH (0.1 N) per 100 mL of juice, was determined according to IFU method n. 30.

The enzyme determination of D-isocitric D-L lactic acids was made according to IFU methods n. 53 and 54. For ethanol, glucose, fructose and sucrose were used enzymatic methods established by IFU n. 55; n. 55, and n. 56, respectively. The determination of the total pectins was achieved by IFU method n. 26. The pectin content was expressed in mg/L of galacturonic acid determined colorimetrically.

The flavonoid determination in the juices by RP-HPLC has been carried out according to IFU method n. 58, with few modification according to Cautela et al. [5]. The HPLC analyses were performed by a Surveyor instrument (Thermo Fisher Scientific, Italy) connected with in-line diode-array (PDA) on a Luna C18 Column (Phenomenex, USA).

Sodium, potassium, magnesium and calcium contents were determined by by inductively coupled plasma–optical emission spectrometry (ICP–OES) using an ICP OPTIMA 2000 instrument from Perkin-Elmer (Monza, Italy) according to IFU method n. 33. Arsenic and heavy metals were determined by graphite furnace atomic absorption spectrometry (GF–AAS) using an AAnalyst 600 spectrometer (Perkin-Elmer, Monza, Italy) interfaced to an AS800 autosampler.

2.4. Statistical Analysis

Each juice sample was analysed in triplicates and the mean concentration of each compound was calculated and expressed in mg/L or mg/kg of product.

3. Results and Discussion

The 'Chinotto' juice extracted was amber yellow, with shades tending to orange yellow; further it was pulpy, with a sweet taste but a bitter aftertaste. The results of chemical composition analyses of 'chinotto' juice were reported in Table 1.

3.1. Absolute Quality Requirements Parameters

The chinotto juice show an average TSS value residue of 10.0 °Brix with the centrifugable pulps made up of about 6.2% *v/v*. As described in Table 1, the relative densities of the juice was 1.0400. Other environmental, hygienic, and industrial requirements are inside the range of values established in the reference guideline for other citrus juices. Heavy metal elements are below the method detection limit values.

Furthermore, the parameters of D-L lactic acid were not exceeding the maximum permitted content of 0.5 g/L for an orange juice according to the AIJN Code of Practice [3].

Table 1. Quality requirements, criteria, and parameters for assessment of identity and authenticity of Chinotto juice.

	Unit	Mean ± SD	Range of Variation
Absolute quality requirements			
<i>1. Industrially agree upon requirements</i>			
Rel. density 20/20		1.0400 ± 0.01	1.0355–1.0484
Soluble solids	°Brix	10.0 ± 1.5	8.9–12.0
<i>2. Hygiene requirements</i>			
Ethanol	g/L	< 0.01	
D-lactic acid	g/L	0.23 ± 0.04	0.19–0.29
L-lactic acid	g/L	0.11 ± 0.03	0.09–0.15
<i>3. Environmental requirements</i>			
Arsenic (As)	mg/L	<0.005	
Lead (Pb)	mg/L	<0.01	
Mercury (Hg)	mg/L	<0.01	
Cadmium (Cd)	mg/L	<0.01	
<i>4. Compositional requirements</i>			
Ascorbic acid	mg/L	921 ± 91	867–997
Other quality parameters			
Titratable acidity at pH 8.1	g/L	8.1 ± 4.5	3.6–12.5
Formol number	mL NaOH 0.1 N/100 mL	20.9 ± 6.1	14.8–30.5
D-isocitric acid	mg/L	131 ± 18	121–152
Glucose	g/L	25.2 ± 1.6	23.9–28.2
Fructose	g/L	28.0 ± 1.9	26.0–30.8
Sucrose	g/L	32.1 ± 2.3	28.8–36.1
Total pectins	mg/L	247 ± 80	161–345
Sodium (Na)	mg/L	11 ± 5	8.1–18
Potassium (K)	mg/L	3042 ± 165-	2780–3250
Magnesium (Mg)	mg/L	83 ± 11	62–98
Calcium (Ca)	mg/L	115 ± 31	80–148
neoeriocitrin	mg/L	475 ± 82	372–582
eriocitrin	mg/L	35 ± 13	3–45
naringin	mg/L	832 ± 147	672–989
neohesperidin	mg/L	723 ± 93	630–823

The ascorbic acid concentration was quite higher than orange juice average content [3], with a mean concentration of about 900 mg/L, and a variability included between 867 and 997mg/L (Table 1).

3.2. Criteria Relevant to the Evaluation of Identity and Authenticity

The acidity of chinotto juice, expressed as the content of anhydrous citric acid in g/L, was less than that orange juice (AIJN) [3] showing an average value of 8.1 g/L above all samples analyzed and ranging from 3.6 to 12.5 g/L. Among the other organic acids, the content of D-isocitric acid did not exceed 150 mg/L in all samples, reaching a maximum value of 131 mg/L.

The number of formol is the index that reflects the amount of free amino acids and is often used to ascertain the genuineness of a juice. The data in Table 1 show an high variability of concentration because this parameter is affected by the harvest time of fruits. The formol number varied from 14.8 for the juice obtained from unripe fruits, up to 30.5 for those picked in March. The central value was 29.9 mL of 0.1 N NaOH per 100 mL of juice.

The more representative sugars of 'Chinotto' juice were sucrose with an average concentration of about 32 g/L, followed by fructose with a mean content of 28 g/L. The glucose content was about 25 g/L. The amount of free sugars of chinotto juice was comparable to that reported in literature for orange juice [3].

In citrus juices, pectin was one of the major components of the suspended cloud material that confers desirable appearance, texture, and flavor [6]. Total pectin content varies depending on juice extraction techniques used so in this study, since a manual citrus squeezer was employed, this parameter not exceeds 350 mg/L.

The main mineral present in chinotto juice was potassium (K), with a significant value equal to 3042 mg/L. The concentration levels of calcium (Ca) and magnesium (Mg) ranging from 62 to 148 mg/L, respectively; while sodium (Na) content was below 19 mg/L for all juice samples.

Flavonoids are commonly used as chemotaxonomic markers of juices because evaluate the quality and authenticity, varying their flavonoidic profile from juice to juice [5]. Flavanones usually occurred as O-glycosyl derivatives, with the sugar moiety bound to the aglycone hydroxyl group at either C7 or C3. Among these compounds, the O-diglycosides were a dominant category and their structures were usually characterized by the linkage of either neohesperidose or rutinose to the flavonoid skeleton. Three main flavanones (naringin and neosperidin and neoeriocitrin) in chinotto juice are flavanone-7-O-neohesperidosides. The bitterness caused by flavanone-7-O-neohesperidosides was often referred to as 'primary' bitterness, while flavanone-7-O-rutinosides were tasteless [7]. Naringin is the most abundant flavonoid with a value of 832 mg/L, and with a variability range between 672 and 989 mg/L. Likewise the neohesperidin was present at similar amount of naringin, showing a mean value of 723 mg/L. The neoeriocitrin content ranged from 372 to 582 mg/L, with an average content of 475 mg/L. The impact of eriocitrin, a flavanone-7-O-rutinosides, respect to the other flavonoids was negligible reaching an average value of 35 mg/L.

It is interesting to note that although the content of organic acids and sugars is similar to that of orange juice, rutinoside flavonones are absent in the chinotto juice, but it is rich in neospereridosidic flavonoids which give the product its characteristic sour taste.

4. Conclusions

The study on the chemical-physical characterization of chinotto juice here presented set down the earliest outlines of quality and authenticity for this product. These outcomes could represent a starting point for defining guidelines of food and safety quality for chinotto juice, preventing any potential adulterations or frauds.

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