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Chemical Composition of Chinotto Juice †

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Abstract: *Citrus* × *myrtifolia* (Rafinesque) fruits are commonly used to produce the popular Italian beverage 'Chinotto'. The *C. myrtifolia* plant comes from Asia, like most *Citrus* spp., but is currently spread across Mediterranean countries including Italy, mostly Liguria and Sicily. The fresh juice obtained by squeezing ripe fruits of Chinotto was investigated with the aim of drawing 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, and 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 fraud.

Keywords: *Citrus myrtifolia*; Chinotto; flavonoids; chemical composition

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1. Introduction

Citrus × *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 to southern China, its origin has not been exactly ascertained. The plant has been cultivated for centuries in France and Italy. In Italy, the production of Chinotto fruits is concentrated in the southern regions, Sicily and Calabria, as well as on the Ligurian coast.

The unripe fruits look like small green aromatic tangerines, while mature fruits are bigger and orange. The flesh is bitter and sour, and it can be 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 fruit 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 was to help fill the knowledge gaps in terms of the authentic composition for Chinotto juice, obtained from the edible part of fruits via mechanical processes. Hence, it will be possible to establish some reference guidelines for this product to protect consumers from food fraud by 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 kits for sugars (glucose, fructose, and saccharose) organic acids (D-isocitric and D,L-lactic acids), 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 the 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 into 50 mL plastic containers and immediately stored at −20 °C until analysis.

2.3. Analytical Reference Methods

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

Total soluble solids (TSS), expressed in °Brix, were determined by means of measuring the refractive index at 20 °C using a digital refractometer (Mettler-Toledo S.p.A; Milan Italy) according to IFU method No. 8. Relative density at 20 °C was determined using IFU method No. 1. Titratable acidity was measured according to IFU method No. 3. Formol number, expressed as mL of NaOH (0.1 N) per 100 mL of juice, was determined according to IFU method No. 30.

The enzyme determination of D-isocitric and D,L-lactic acids was carried out according to IFU methods No. 53 and 54. Ethanol, glucose, fructose, and sucrose were determined using enzymatic methods IFU No. 55, No. 55, and No. 56. The determination of total pectins was achieved using IFU method No. 26. The pectin content was expressed as mg/L of galacturonic acid determined colorimetrically.

Flavonoid determination in the juices by RP-HPLC was carried out according to IFU method No. 58, with some modification according to Cautela et al. [5]. The HPLC analyses were performed using a Surveyor instrument (Thermo Fisher Scientific, San Jose, CA, USA) connected to an in-line diode-array (PDA) on a Luna C18 Column (Phenomenex, Castel Maggiore, BO, Italy).

Sodium, potassium, magnesium, and calcium contents were determined by inductively coupled plasma optical emission spectrometry (ICP–OES) using an ICP OPTIMA 2000 instrument from Perkin-Elmer (Monza, Italy) according to IFU method No. 33. Arsenic and heavy metals were determined by graphite furnace atomic absorption spectrometry (GF–AAS) using an AAnalist 600 spectrometer (Perkin-Elmer, Monza, Italy) interfaced to an AS800 autosampler.

2.4. Statistical Analysis

Each juice sample was analyzed in triplicate, 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; furthermore, it was pulpy, with a sweet taste but a bitter aftertaste. The results of chemical composition analyses of 'Chinotto' juice are reported in Table 1.

3.1. Absolute Quality Requirements and Parameters

The Chinotto juice showed an average TSS value of 10.0 °Brix with the centrifugable pulps making up about 6.2% v/v. As described in Table 1, the relative density of the juice was 1.0400. Other environmental, hygienic, and industrial requirements were within the range of values established in the reference guideline for other citrus juices. Heavy metal elements were below the method detection limit values.

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

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

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

	Unit	Mean ± SD	Range of Variation
Absolute quality requirements			
1.Industrially agreed upon requirements			
Rel. density 20/20		1.0400 ± 0.01	1.0355–1.0484
Soluble solids	°Brix	10.0 ± 1.5	8.9–12.0
2. Hygiene requirements			
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
3. Environmental requirements			
Arsenic (As)	mg/L	<0.005	
Lead (Pb)	mg/L	<0.01	
Mercury (Hg)	mg/L	<0.01	
Cadmium (Cd)	mg/L	<0.01	
4. Compositional requirements			
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

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 in orange juice (AIJN) [3] showing an average value of 8.1 g/L across all samples analyzed, 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 formol number is an 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 a high variability as this parameter is affected by the harvest time of fruits. The formol number varied from 14.8 for the juice obtained from unripe fruits 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 the literature for orange juice [3].

In citrus juices, pectin is 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; accordingly, in this study, since a manual citrus squeezer was employed, this parameter should not exceed 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) ranged 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 they allow evaluating quality and authenticity, whereby the flavonoidic profile varies from juice to juice [5]. Flavanones usually occur 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. The three main flavanones (naringin, neosperidin, and neoeriocitrin) in Chinotto juice are flavanone-7-*O*-neohesperidosides. The bitterness caused by flavanone-7-*O*-neohesperidosides is often referred to as 'primary' bitterness, while flavanone-7-*O*-rutinosides are tasteless [7]. Naringin was the most abundant flavonoid with a value of 832 mg/L (672–989 mg/L). Likewise, neohesperidin was present at a similar amount (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*-rutinoside, was negligible with respect to the other flavonoids, reaching an average value of 35 mg/L.

It is interesting to note that, although the content of organic acids and sugars was similar to that of orange juice, rutinoside flavonones were absent in the Chinotto juice, which was instead rich in neospereridosidic flavonoids, giving the product its characteristic sour taste.

4. Conclusions

The physicochemical characterization of Chinotto juice presented in this study 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 fraud.

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