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Chemical composition of *kinkan* orange and citrus fruits

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Abstract

Kinkan orange (*Fortunella margarita*), commonly known as "golden orange", is originally from Asia and belongs to the genus *Fortunella*. Kinkan orange has some characteristics of citrus fruits, which are the most consumed fruits by the Brazilian population. *Objective:* The study aimed to evaluate the chemical characterization of kinkan orange by comparing the results obtained with data published on the analysis of some citrus fruits of great commercial interest in Brazil, such as *serra-d'água* orange, *pera-rio* orange, ponkan tangerine and tahiti lime. *Methodology:* We evaluated pH, soluble solids, titratable acidity, and content of vitamin C, total phenolics and antioxidant capacity. All tests were performed in triplicate and data were compared by Tukey's test ($p < 0.05$). *Results:* Kinkan orange had the highest content of soluble solids (SS) and low titratable acidity (TA), consequently a high SS / TA ratio, high vitamin C content and the highest content of total phenolics compared to other fruits. *Conclusion:* Kinkan orange has chemical characteristics similar to other citrus fruits such as pH, titratable acidity and content of vitamin C, has excellent palatability and can be considered as a source of total phenolic and vitamin C in the diet.

Key words: Citrus. Vitamin C. Polyphenols. Antioxidant Capacity.

Introduction

Citrus plants of the genus *Citrus* and *Kunquats* of the genus *Fortunella*, which belong to the family *Rutaceae*, are native to Southeast Asia and have phylogenetic branches extending from central China to Japan and from eastern India to New Guinea, Australia and tropical Africa.[1]

Kinkan orange (*Fortunella margarita*), commonly known as "golden orange" is native to Asia, grows in small-sized trees, blossoms in spring and summer and fruits appear mainly in autumn.[1] It is a plant that can be cultivated for ornamental purposes in gardens and indoors.[2]

Although it belongs to the genus *Fortunella*, kinkan orange has some characteristics of the citrus fruits, such as aroma and flavor, but has a smaller number of segments and the peel is easy to digest.[2] It can be eaten raw and in whole, except the seeds. The peel is soft, sweet and has a typical aroma, mainly due to the presence of flavonoids and terpenoids. This fruit can be used in the preparation of syrups, sauces or jams, added to fruit salads, preserved in whole in sugar syrups or used as decoration.[3,4]

Organic acids, sugars and phenolic compounds are among the major compounds of citrus fruits, which are important factors of quality, once phenolic compounds has antioxidant properties, and the sugars the flavor attributes.[5] Citric and malic acids are the main organic acids components of citrus fruits. Soluble solids and juice sweetness are directly related to the fruit sugar quality.[6]

With regard to the vitamins present in citrus fruits, vitamin C is predominant,[7] and is found with values ranging from 50 to 100mg of ascorbic acid /100g.[8] Vitamin A and vitamins B complex, as well as minerals, especially calcium, potassium, sodium, phosphorus and iron are other key components.[9]

Citrus fruits are the most consumed fruits by the Brazilian population. There are reports of annual *per capita* consumption of 5.59Kg of orange, 0.565Kg of lime and 1.17Kg of tangerine.[10] There are no data on the consumption of kinkan orange, but it is known that in Brazil it is largely cultivated, particularly in the southeast region of the country.[11]

The Brazilian Table of Foods Composition[12] provides information on the chemical composition of various citrus fruits, but there are no scientific data on the chemical composition of kinkan orange. Therefore, the present study aimed to evaluate the chemical characteristics of kinkan orange (*Fortunella margarita*), comparing the results obtained with data published on the analysis of some citrus fruits largely marketed in Brazil, such as *serra-d'água* orange (*Citrus sinensis* (L.) Osb.), pera-rio orange (*Citrus sinensis* L. Osbeck), ponkan tangerine (*Citrus reticulata* Blanco) and tahiti lime (*Citrus latifolia* Tanaka).

Materials and methods

Sampling

The fruits used for analyses were kinkan orange, ponkan tangerine, *serra-d´água* orange, *pera-rio* orange and tahiti lime, *in natura*. The experiment was conducted between June and November 2011, and all fruits were obtained from the Central Market of Belo Horizonte (Belo Horizonte-MG).

Samples characterization

Medium-sized varieties were used in the experiment, with average weight of 50g of kinkan orange, 120g of *serra-d'agua* orange, 124g of *pera-rio* orange, 154g of ponkan tangerine, and 100g of tahiti lime. To perform the analyses, we used the whole kinkan orange (except seeds) and the juice of the other fruits. Sampling was built at random, and medium-sized samples of each variety were used. To perform the analyses, the juice from each sample was extracted manually and then homogeneized.[13]

Physicochemical analyses were carried out on the samples and consisted of the following variables: pH value, determined by digital PHmeter, Digimed brand, model D.M. – 20; total soluble solids (°Brix), analyzed by Baush & Lomb Optical Company refractometer, model ABBE-3L; total acidity (g of citric acid/100ml of juice), determined by titration with 0.1 N of sodium hydroxide solution and phenolphthalein as indicator.[13,14]

Determination of vitamin C content

Determination of vitamin C was performed according to Yurena et al.[7] The samples were frozen at -70°C until the time of analysis. The samples were ground in a blender. 0.5g of sample was weighed and mixed with 2.5ml of the acid-base extraction solution (3% of metaphosphoric acid and 8% of acetic acid). The mixture was homogenized at 18,000g (on ice and in the dark), for one minute and then centrifuged at 9,000g (cooled to 4°C) during 20 minutes. We repeated this procedure twice, and the two resulting supernatants were mixed. All analyses were carried out three times. After repeating the procedures and the supernatant were mixed, the sample was titrated with Tillmans' reagent.[13] Various precautions were taken to perform all operations under reduced light and a temperature of 4°C. In addition, in order to stabilize vitamin C in the acid extraction solution, it was added 0.099g of 2,6-butylated hydroxytoluene (BHT) at 1mM[7].

Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu's method.[15] A standard curve of gallic acid was produced at concentrations of 0 to 0.5mg/ml. 50μl of the pre-homogenized sample was added to 350μL of methanol, followed by the addition of 250μl of the Folin-Ciocalteu's reagent. Next, 3ml of water and 1ml of sodium carbonate were added, the solution was stirred in a vortex mixer, and the mixture was allowed to stand for one hour protected from light. The samples absorbance was measured at 750nm. The results were expressed in milligrams of gallic acid.[15]

Antioxidant capacity

The antioxidant activity was determined by the radical scavenging activity method, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, adapted.[16] The samples were diluted in ethanol (0.8 g of fruit/25 mL of ethanol). In a test tube, 500μl of the diluted sample were mixed with 300μl of the DPPH solution (0.5mM) and 3ml of ethanol. The samples were allowed to stand in the dark and room temperature for 45 minutes. The control was prepared according to the procedure described above, but without addition of the sample. Absorbance was measured at 517nm.

Statistical design

All analyses were performed in triplicate. The statistical software used was *GraphPad Prism* 5.0. Analysis of variance was made with *post hoc* Tukey's test ($p<0.05$), and to verify the correlation between the variables, the Pearson's correlation test was used.

Results

Table 1 shows the results relating to pH values, soluble solids, titratable acidity and soluble solids/titratable acidity (SS/TA) ratio of the fruits analyzed. According to the analyses, the kinkan orange had the highest content of soluble solids in relation to the other fruits, high SS/TA ratio in relation to the *pera-rio* orange, ponkan tangerine and lime, and intermediate pH and titratable acidity values.

Table 1. Values of pH, soluble solids, titratable acidity and SS/TA ratio of the fruits analyzed. Belo Horizonte-MG, 2011.

Sample	pH	Soluble solids (ºBrix)	Titratable acidity (g citric acid / 100 ml of juice)	SS/TA
Kinkan orange	4.22 ± 0.03[b]	21.1 ± 0.10[a]	1.14 ±0.16[b]	18.89 ± 2.74[b]
Serra d'água orange	5.55 ± 0.04[a]	15.2 ± 0.20[b]	0.82 ± 0.50[b]	29.27 ± 17.60[a]
Pera-rio orange	4.04 ± 0.02[c]	9.40 ± 0.40[c]	5.58 ± 0.98[a.b]	1.73 ± 0.23[d]
Ponkan tangerine	3.55 ± 0.01[d]	14.4 ± 0.40[b]	5.39 ± 0.11[a.b]	2.67 ± 0.13[c]
Tahiti lime	2.56 ± 0.02[e]	8.35 ± 0.35[c]	9.80 ± 1.70[a]	0.87 ± 0.12[e]

Mean ± standard deviation of two replicates.

Different letters in the columns mean difference at a significance level of p<0,05.

Table 2 presents the results relating to vitamin C and total phenolic contents, and the antioxidant capacity of the fruits analyzed. Results indicate that the kinkan orange had a high content of vitamin C and the highest total phenolic content compared to the other fruits. The antioxidant capacity was similar to the one found for *serra-d'água* orange, ponkan tangerine and tahiti lime.

Table 2. Vitamin C, total phenolic contents, and antioxidant capacity of the fruits analyzed. Belo Horizonte-MG, 2011.

Sample	Vitamin C (mg ascorbic acid/ 100 ml of juice)	Total phenolics (mg gallic acid/g of fruit)	Antioxidant capacity (%)
Kinkan orange	86.45 ± 6.65[a]	0.09 ± 0.0010[a]	52.99 ± 8.83[b.c]
Serra d'água orange	63.18 ± 3.33[a.b]	0.04 ± 0.0002[c]	55.68 ± 6.15[b.c]
Pera-rio orange	53.20 ± 6.65[b]	0.05 ± 0.0004[b]	119.08 ± 16.56[a]
Ponkan tangerine	73.15 ± 0.00[a.b]	0.03 ± 0.0008[d]	31.70 ± 3.31[c]
Tahiti lime	56.52 ± 3.33[b]	0.05 ± 0.0016[b]	86.28 ± 3.00[a.b]

Mean ± standard deviation of two replicates.

Different letters in the columns mean difference at a significance level of p<0.05.

Table 3 presents the correlation between the pH value, titratable acidity and soluble solids of the fruits. A strong negative correlation was found between the pH and titratable variables and between titratable acidity and soluble solids contents.

Table 3. Correlation between the variables "pH", "titratable acidity" and "soluble solids content" of the fruits analyzed. Belo Horizonte-MG, 2011.

		Pearson's correlation
pH	Titratable acidity	-0.896294932*
pH	Soluble solids	0.502650643ns
Titratable acidity	Soluble solids	-0.828230191*

* Significance level = $p<0.05$; ns= not significant

Table 4 presents the correlation between the variables vitamin C, total phenolic content and antioxidant capacity. There was a moderate negative correlation between the antioxidant capacity and vitamin C content.

Table 4. Correlation between the variables "antioxidant capacity", "vitamin C" and "total phenolic content" of the fruits analyzed. Belo Horizonte-MG, 2011.

		Pearson's correlation
Antioxidant capacity	Vitamin C	-0.740596*
Antioxidant capacity	Total phenolics	0.084740ns

* significance level = $p<0.05$; ns= not significant

Discussion

Kinkan orange presented intermediate pH values (4.22 ± 0.03) in the fruits analyzed and determined pH values within the range of 3.20 ± 0.01 to 5.43 ± 0.06, as reported by Couto & Canniatti-Brazaca,[16] after analyzing other orange varieties. Higher pH values are preferred for *in natura* consumption,[17] suggesting that kinkan orange is a fruit indicated for *in natura* consumption.

Determination of titratable acidity is used to demonstrate the acidity level in the solution, i.e., the amount of citric acid contained in 100ml of solution.[16] Kinkan orange presented a similar titratable acidity content to that found in some fruits analyzed (1.14g citric acid/100ml of juice ± 0.16), indicating its similarity with such citrus fruits.

Soluble solids indicate the fruit sweetness in directly proportional amount, i.e., the higher the level of soluble solids, the higher the fruit sweetness.[6] According to the results found, kinkan orange presented the highest level of soluble solids (21.1ºBrix ± 0.10) and, consequently, a higher degree of sweetness in relation to the other fruits analyzed in the study.

One of the most important parameters to report on the fruits palatability is the ratio between the soluble solids content and titratable acidity (SS/AT). In general, Brazilian consumers prefer a high SS/AT ratio.[17] According to the results found, kinkan orange has a high SS/AT ratio (18.89 ± 2.74), indicating that the fruit has good palatability.

Vitamin C is the main vitamin found in citrus fruits and can be considered a characteristic of these foods.[9] It is known that this vitamin plays a role in the cellular redox processes, prevents scurvy, it is important for the biosynthesis of catecholamines, aids in the defense of the body against infections, is vital for the integrity of the blood vessels, and indispensable to the formation of collagenous fibers. However, it is not synthetized by the body, which makes the intake of this vitamin essential to human health.[18] In citrus fruits, the level of vitamin C is generally found in the range of 50 to 100mg/100ml of juice.[8] According to the results found, kinkan orange presented a high content of vitamin C (86.45mg/100g), indicating that it has a characteristic that is common in citrus fruits.

Phenolic compounds are secondary metabolites naturally present in fruits and vegetables, and its importance is often related to the prevention of some diseases. It plays a key role on the free radical-scavenging action, which allows to reduce the incidence of diseases like atherosclerosis, cataracts, cancer and chronic inflammations.[19] Kinkan orange presented a high total phenolic content (0.09 ± 0.0010mg gallic acid/g of fruit), compared to the other fruits under analysis, which allows us to affirm that this fruit can be consumed as a way to prevent some diseases, within the context of a healthy diet.

Several studies have pointed vitamin C as one of the major antioxidants of fruits;[7,19,20] however, this vitamin contributes with only 0.4% of the antioxidant capacity.[21] On the other hand, corroborating the finding of this study, Couto & Canniatti-Brazaca[16] did not find a correlation between the level of vitamin C and the antioxidant capacity of the fruits analyzed. This finding suggests that the higher antioxidant capacity of the kinkan orange can be justified by other bioactive compounds present in the fruit.[21] Likewise, in the present study, the antioxidant capacity was not associated with the total phenolic content, suggesting that other compounds that were not investigated, such as anthocyanins and carotenoids may be responsible for the fruits antioxidant capacity[22].

Conclusions

Kinkan orange has chemical characteristics similar to the ones found in citrus fruits, such as the pH value, titratable acidity and vitamin C content. It has good palatability and can be considered a source of total phenolics and vitamin C in the diet.

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