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Physicochemical evaluation of whole orange juice commercialized in Brazil

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Abstract

Background: Brazil is the world leader in growing oranges and most of this production is destined for juice production. The quality of the juices is very important to consumers that intend to benefit of this kind of beverage.

Aim: In this context, the aim of this work was to evaluate the physicochemical parameters as well as the presence of vitamin C, anthocyanins and carotenoids in different brands of whole orange juice.

Methods: We have studied seven commercial brands of whole orange juice that were denominated 1A, 1B, 2, 3, 4, 5, 6A, 6B and 7. Evaluation of pH, Total soluble solids, Total carbohydrates content, *Ratio*, Ascorbic acid, Anthocyanins and Carotenoids were performed.

Results: According to the Brazilian identity standards for juices, only the whole orange juice of brand 1A was not in accordance with the regular standards. The Brazilian Legislation determines that orange juice presents at least 25.5 mg of ascorbic acid in 100 mL of juice, so the brands 2, 5, 6A and 6B possess values below the minimum established by Legislation. Furthermore, our results showed significant differences between the brands. Significant differences were found for the levels of anthocyanins and carotenoids among the brands. According to Brazilian Legislation, we may say that only brands 3, 4 and 7 meet all the legal requirements for the commercialization of orange juice.

Conclusion: We suggest that parameters such as anthocyanins and carotenoids should be considered once they promote benefits to human health as well we suggest a more rigorous inspection, thus benefiting consumers and avoiding misunderstandings when choosing and acquiring the product.

Keywords: orange juice; vitamin C; anthocyanin; carotenoid

1. Introduction

Brazil is the world leader in growing oranges and most of this production is destined for juice production. Currently the country is the largest exporter of orange juice, with a total of 1.103.126 tons of the beverage exported in 2015. The average of consumption per capita consumption is 12.4 liters/year, behind, United States and European Union countries [1, 2].

Brazilian legislation defines as an integral orange juice the beverage that is non-fermented, undiluted, without added sugar, sweeteners and dyes and with less than 0.5% of ethyl alcohol, produced with healthy and mature oranges (*Citrus sinensis*) by means of appropriate process and subjected to treatment that ensures its conservation until consumption. This legislation is governed by the Ministry of Health through the National Sanitary Surveillance Agency (ANVISA, Brazil) and the Ministry of Agriculture, Livestock and Food Supply (MAPA, Brazil) [1, 3].

Authors believe that the consumption of beverages with nutritive values allied to a pleasant taste tends to increase in the population and the orange juice may be an interesting choice due to its nutritional characteristics that include the presence of vitamin C, minerals, carbohydrates and antioxidants [4, 5].

The daily consumption of orange juice contributes to the reduction of the incidence of hypertension in adult men, may have a positive impact on lipid metabolism, and reduces the postprandial glycemic and insulinemic responses to typical meal ingestion in men. Authors also have shown that the consumption of orange juice may reduce obesity, probably due to its effectiveness at increasing satiety. It is capable of improving immune system, work as anti-inflammatory and antioxidant. These benefits occur probably to the presence of phytochemicals that may help preventing the development of chronic degenerative diseases such as diabetes, obesity, Metabolic Syndrome and cardiovascular diseases that are among the main causes of death worldwide [6-12].

The quality of the juices is very important to consumers that intend to benefit of this kind of beverage. In this context, the aim of this work is to evaluate the physicochemical parameters as well as the presence of vitamin C, anthocyanins and carotenoids in different brands of whole orange juice.

2. Methods

2.1 Raw materials and analyses

We have studied seven commercial brands of whole orange juice in carton packs (Tetra Pak), glass or plastic container purchased in commercial establishments in the city of Marília – Sao Paulo, Brazil in March 2016. The analyzed brands were denominated 1A, 1B, 2, 3, 4, 5, 6A, 6B and 7. For the brands that produce juices with two different types of orange, letters were used to distinguish them.

pH was evaluated with a digital pH meter with automatic temperature compensation (PG 1800 GEHAKA®). Total soluble solids were expressed in ºBrix and were evaluated with an Abbe refractometer equipped with a digital thermometer (Nova Instruments®). Total carbohydrates content were expressed in % (m/m) using Fehling reaction, and acidity was expressed as % (v/v) of citric acid according to Adolfo Lutz Institute [13]. The *Ratio* was calculated using the ratio of total solids (ºBrix) to citric acid. All the parameters studied were performed in triplicate.

Vitamin C levels were expressed as mg/100mL, and were evaluated according to Terada *et al*. [14]. Anthocyanins were expressed as mgAT/100g and were evaluated according to Lees and Francis [15] with modifications (absorbance performed at 535nm). The determination of carotenoids was performed according to Higby [16] (absorbance performed at 520nm) and expressed in mg/100g of the total carotenoids solubilized in hexane.

2.2 Statistical analyzes

Data of pH, ºBrix, acidity (% v/v), Vitamin C (mg/100mL), Anthocyanins (mg AT/100g), Total carbohydrates (% m/m), *Ratio* and Total carotenoids were evaluated by analysis of variance (ANOVA), complemented with Tukey's test and Student's *t* test, performed by the BioEstat 5.0 statistical software program.

3. Results and Discussion

Table 1 shows the averages and the standard deviation in relation to the physical-chemical parameters of pH, titratable acidity, total soluble solids, total carbohydrates, and *Ratio* of different brands of integral orange juice.

Table 1: Physicochemical analyzes of different brands of whole orange juice.

Brand	pH	Acidity (% v/v)	Total soluble solids (^{0}Brix)	Total carbohydrates (%m/m)	*Ratio*
1A	3.83±0.02 A[1]	0.598±0.009 B	9.80±0.00 A	10.36±0.12 A	16.400±0.258 B
1B	4.10±0.00 C	0.559±0.001 A	10.70±0.20 B	12.20±0.11 C	19.151±0.387 C
2	4.06±0.02 B	0.796±0.003 D	10.17±0.31 A	9.85±0.25 A	12.771±0.436 A
3	4.06±0.01 B	0.520±0.014 A	10.50±0.20 B	10.83±0.12 A	20.210±0.646 C
4	4.09±0.00 BC	0.703±0.029 C	10.90±0.00 B	11.20±0.61 B	15.524±0.645 B
5	3.85±0.02 A	0.647±0.010 C	10.43±0.31 B	10.78±0.09 A	16.126±0.411 B
6A	3.89±0.08 A	0.558±0.002 A	11.40±0.00 C	12.36±0.03 C	20.424±0.070 C
6B	3.94±0.10 AB	0.551±0.002 A	11.27±0.06 C	11.36±0.05 B	20.466±0.093 C
7	3.87±0.01 A	0.585±0.007 AB	11.70±0.10 C	11.41±0.05 B	20.018±0.084 C

[1]Means followed by at least one same letter do not statistically differ.

According to the Brazilian identity standards for juices, the whole orange juice should present levels of total soluble solids at minimum of 10.5^{0}Brix, *Ratio* of 7.0 and ascorbic acid 25.5mg/100mL. For total carbohydrates, the maximum value should range 13.0g/100g. It is possible to see that brand 1A (table 1) is not in accordance with the first parameter [3]. All the other samples are in agreement with the Legislation for these parameters.

The pH values obtained in this study presented an average around 3.85, similar to the values found by Sugai *et al*. [17], in an analysis performed with samples of pasteurized orange juice. Silva *et al*. [18] also found similar results, showing pH values ranging from 3.51 to 4.02. On the other hand, Couto and Canniatti-Brazaca [19] found pH values varying from 3.20 to 5.43 in different varieties of oranges.

Reduced results for pH and high acidity may contribute to better preservation of the product. Although results for pH are not regulated by Brazilian law, it is important in the production of beverages, since it should not be higher than 4.5 to not facilitate the development of *Clostridium botulinum* [20, 21].

The evaluation of the acidity is an indicator of the conservation of a food, and an important parameter of quality. In our study, all the brands presented significant differences for the acidity values expressed in citric acid. The highest value was obtained in brand 2 and the lowest in brand 3. Santos *et al*. [22] postulate that it is possible to find small variations in the total acidity in orange juice due to the different degrees of maturation and varieties of oranges used to produce the juice. The same authors, when analyzing eight different varieties of orange at different maturation stages, found total acidity results similar to those of our study. Venâncio, Martin [23] studied whole orange juice brands and found a result of 22.93% v/v of total acidity, which is close to the average obtained in this work. According to the authors, besides the degree of maturation, the region in which the orange was grown may also interfere with the total acidity value.

The soluble solids present in the fruit pulp contain sugars and organic acids responsible for the acceptance of the product by consumers (Lima *et al*. [24]. Soluble solids content is a parameter that has been used as an indicator of fruit quality. It is of great importance both for *in natura* consumption and for industrial processing, since high levels of these constituents in the raw material imply in lower sugar addition, shorter water evaporation time, lower energy expenditure and higher product yield [25]. A study by Ortiz [26] showed that the total soluble solids content in orange juice ranged 11.00 and 11.46 °Brix. Also Tazima *et al*. [27] when analyzing oranges *in natura*, obtained results between 10,65 and 11,16 °Brix.

The values of total carbohydrates indicated in table 1 showed significant differences among the brands, with variations from 9.85% to 12.36% m/m. Our results are inferior to those found by Todisco, Clemente, Rosa [28], who analyzed orange *in natura* for sixty days in two different temperatures: 25°C and 7°C. In both temperatures the lowest value was 13.27% m/m. This difference may have occurred because the oranges used in the production of the juice are at a higher maturation stage or because the orange varieties are different from those used by the authors. In a study by Cruz *et al*. [29] with fresh mandarins showed a value of approximately 10.5% w/w of total carbohydrates, similar to our results.

Table 1 also shows the results for the *Ratio*. There are significant differences between the brands of orange juice studied in our work, however, all of them obey the standards of the current Brazilian Legislation. *Ratio* is based on the values of ºBrix and titratable acidity and is a parameter used to identify the degree of maturation and flavor of the fruits, so the industries have greater control in their productions and standardizations, always maintaining the same quality of their products. It is an important parameter used by industries that produce juices because it is indicative of the balance among sweetness and fruit acidity [30, 31]. The differences among the brands may occur due to the differences among the acidity of the juices, once the *Ratio* is inversely proportional.

Figueira *et al*. [32] carried out physicochemical analyzes on orange juices and obtained results ranging from 14.95 to 19.45 and an average of 16.33, similarly to our findings. The authors also analyzed the *Ratio* of concentrated orange juice and found values ranging from 12.66 to 15.52. They also reported that consumers of citrus juices prefer products with a *Ratio* ranging from 15 to 18, confirming the assumption that the beverage industries manufacture their products with balanced proportions between the amount of sugar and the titratable acidity. Couto, Canniatti-Brazaca [19] studied different varieties of oranges and found values ranging from 6.67g/100g to 17.81g/100.

Table 2 shows the mean and the standard deviation of vitamin C, anthocyanins, and total carotenoids of the different brands of integral orange juice.

Table 2: Vitamin C, Anthocyanins and Total carotenoids in the different brands of whole Orange juice.

Brand	Vitamin C (mg/100m)	Anthocyanins (mgAT/100g)	Carotenoids (mg/100g)
1A	26.67±1.37 AB[1]	0.083±0.000 A	0.001±0.000 A
1B	27.07±0.98 B	0.785±0.066 B	0.016±0.004 B
2	23.23±0.64 A	0.601±0.133 B	0.006±0.001 A
3	32.53±1.64 C	0.557±0.128 B	0.003±0.001 A
4	33.70±2.95 C	0.417±0.000 B	0.023±0.012 B
5	21.00±1.65 A	2.978±0.064 C	0.003±0.003 A
6A	23.67±0.42 A	1.113±0.241 B	0.010±0.003 AB
6B	24.63±2.55 A	0.696±0.337 B	0.065±0.012 C
7	26.30±1.31 A	2.254±1.312 BC	0.005±0.001 A

[1]Means followed by at least one same letter do not statistically differ.

The Brazilian Legislation determines that orange juice presents at least 25.5 mg of ascorbic acid in 100 mL of juice [32], so the brands 2, 5, 6A and 6B possess values below the minimum established by Legislation. Furthermore, our results showed significant differences between the brands. This must have occurred due to the way of extraction of the juice, the type of packaging and the conservation. Ascorbic acid is very sensitive to light, oxygen and high temperatures, easily degrading when in contact with these factors. It is more vulnerable to degradation during processing steps and storage when compared to other compounds and it may play an important role as an indicator of the nutritional quality of products derived from fruits [23, 33, 34].

When analyzing the orange *in natura*, Caputo [35] found values of ascorbic acid between 30 and 50mg/100mL that are superior to those found in our results. Silva *et al*. [36] conducted a study evaluating the degradation of ascorbic acid in orange juice and found values of 42.22mg/100mL.

The Brazilian Legislation stipulates that a type of food is declared high in some vitamin if it reaches at least 30% of the Recommended Daily Intake (RDI). For products that are called to be source of some vitamin, it must contain at least 15% of the IDR (reference per 100 mL for liquid food). Based on the IDR found in the label of the juices (45mg/day), we may say that all the brands are rich in vitamin C [37-38]. On the other hand, The Dietary Reference Intakes (DRI) of this vitamin is 45 mg/day for children (9 to 13 years old). For ages between 14-18 years old, DRI are respectively 75 mg/day and 65m/day for man and woman. For ages over 19, DRI for man is 90mg/day and for woman is 75mg/day [39]. If we consider these values, in order to satisfy the required amounts of vitamin C, the intake of the juices may be higher than the postulated on the labels.

Our results did not show significant differences between brands 2, 5, 6A, 6B and 7, as there was no difference between 3 and 4. According to table 3, the juices of brands 1A, 1B, 4, 6A and 6B presented values of vitamin C below to that one declared on the packaging. In the labels of the brands 3, 5 and 7, the vitamin C content was above that mentioned in the nutritional information. It should also be noted that brands 1A and 1B are not in accordance with the Resolution 360 of December 2003, which states a tolerance of plus or minus 20% in relation to the nutrients declared on the label [40]. For brand 2, a value of 46.46 mg/200 mL of vitamin C was found, but there was any information about this parameter on the label. Cunha *et al*. [41] found values of 34.87mg of vitamin C in orange juice. Ywassaki, Canniatti-Brazaca [42] studied the presence of ascorbic acid in juices in different times after processing and found values higher than those found by Cunha *et al*. [41]. These findings are superior than we found in or results.

Table 3: Comparison of the levels of ascorbic acid showed on the labels of the juice and the results obtained in our analysis.

Brand	Vitamin C in the labels (mg/200mL)	Results of vitamin C (mg/200mL)
1 A	75mg	54.34mg
1B	75mg	54.14mg
2	unavailable	46.46mg
3	64mg	65.06mg
4	78mg	67.40mg
5	30mg	42.00mg
6 A	72mg	47.34mg
6 B	72mg	49.26mg
7	45mg	52.60mg

Vitamin C is related to several benefits for human beings as production of collagen, noradrenalin, serotonin, and steroid hormones. It is associated with the modulation of the immunity system and may work reducing inflammation and oxidative stress [43-44].

Table 2 shows results for anthocyanin ranging from 0.083 to 2.978 mgAT/100g. This parameter, as well as vitamin C, is a parameter of fundamental importance, since they bring health benefits, however, Brazilian Legislation does not determine reference values for anthocyanins in orange juice and there are no DRI for this compound. Anthocyanins belong to the class of flavonoids that are pigments responsible for the blue, red, violet and purple coloration of many species of plants. They may be associated to various benefits to human health, such as antioxidant, anti-inflammatory, and inhibition of LDL oxidation [45]. The differences in the anthocyanin content observed among the brands may be due to the stability of the same depending on several factors, such as temperature, pH, light, oxygen, enzymatic degradation and interactions of food components [46-47]. Anthocyanins are very sensitive to high temperatures and some of the juices analyzed underwent pasteurization. Brands

1A, 1B, 5, 6A, 6B and 7 do not use pasteurization, and according to our results they present the highest anthocyanin content.

Carotenoids are a group of pigments responsible for colors from yellow to red in fruits and other vegetables. When consumed, they are converted to vitamin A that performs various functions in our body acting as antioxidant, anti-inflammatory, and improvement of immune function, required for regular vision, gene expression, reproduction, and embryonic development [48-50]. The brand that most differed from the others in the analysis of carotenoids was 6B, this may have occurred because the juice is produced from a variety of orange different from the others, according to the manufacturer, it is produced from red orange.

DRI for vitamin A is of 600μ/d for ages of 9 to 13 years; 700 μ/d for ages above 14 years (woman) and 900 μ/d man [39]. Based on this we may say that, according to the Brazilian Legislation, the brands of orange juice evaluated in our work are not source or rich in Vitamin A.

In recent years, a search for healthier products has been observed and orange juice may be a good alternative of acquiring these products. Nevertheless, the consumer should be aware of the standards set out in the labeling since not all brands meet the expectations of eating a good quality product. Anthocyanins and carotenoids, as well as vitamin C, play important role in human health. Due to this, it would be interesting to consider the inclusion of these parameters in the labels of the juices.

4. Conclusion

According to the rules established by the current Brazilian Legislation on beverages and labeling, we may say that only brands 3, 4 and 7 meet all the legal requirements for the commercialization of orange juice. It was also noteworthy to say that the Legislation is flawed for the parameters of pH, anthocyanins and carotenoids. Thus, we suggest that these parameters should be considered once they promote benefits to human health as well suggest a more rigorous inspection, thus benefiting consumers and avoiding misunderstandings when choosing and acquiring the product.

5. Disclosure

Authors declare no conflict of interests

6. References

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