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Bioactive compounds, antioxidant activity, and maturation effects in bitter and sweet orange juices

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Abstract

Bitter oranges (*Citrus aurantium*) and sweet oranges (*Citrus sinensis*) are commercially important citrus fruits with distinct uses and characteristics. Bitter oranges, primarily used as a flavoring agent, are rich in bioactive compounds, while sweet oranges dominate global citrus juice production. This study evaluated bitter orange juice at three maturation stages, comparing its physical, chemical, and bioactive properties with pasteurized pear orange juice (*C. sinensis*). Parameters assessed included water activity, pH, total titratable acidity, total soluble solids, maturation index, vitamin C content, total phenolic (TP) content, total flavonoid (TF) content, and antioxidant activity. The results showed that the vitamin C content in bitter oranges decreased with ripening but remained comparable to pear orange juice in the ripe stage. TPs and TFs were highest in pasteurized pear orange juice, reflecting the stability of these compounds during thermal processing. The antioxidant activity varied significantly across bitter orange maturation stages, with intermediate fruits demonstrating the strongest capacity. However, the maturation stage had limited influence on the antioxidant activity compared to pear orange juice. Bitter orange juices, despite being less commercially prominent, offer distinct bioactive advantages and antioxidant potential. Future research should explore optimizing maturation and processing methods to enhance the nutritional and functional properties of bitter orange juices.

Keywords: vitamin C; 2,2-diphenyl-1-picrylhydrazyl; flavonoids; phenolics.

Practical Application: Bitter orange juice offers rich bioactives, strong antioxidants, and stage-dependent benefits.

1 INTRODUCTION

The bitter orange (*Citrus aurantium*), a member of the Rutaceae family, thrives in tropical and subtropical regions and is native to East Africa, Arabia, and Syria. This tree typically grows to a height of approximately 5 m and produces fruits with significant commercial value. Commonly referred to as Seville orange or sour orange, it is primarily used as a flavoring and acidifying agent in foods (Karabiyikli et al., 2014; Mannucci et al., 2018; Wen et al., 2021).

The sweet orange (*Citrus sinensis*) is the most widely cultivated and consumed citrus species globally, accounting for nearly 50% of the total citrus production (Seminara et al., 2023). Native to Southeast Asia, specifically the region between China and India, sweet oranges dominate Brazil's citrus industry. The pear orange variety is predominantly grown in Brazil, serving both the domestic and export markets for fresh fruit and juice production (Bastos et al., 2017).

Antioxidants are compounds that inhibit or significantly slow the oxidation of other substances. Examples include phenolic compounds, antioxidant enzymes, iron ligands, and transport proteins (Parcheta et al., 2021). Common synthetic antioxidants used to enhance food stability include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Lourenço et al., 2019). However, concerns about potential health risks associated with synthetic antioxidants have prompted stricter regulations and growing interest in natural alternatives (Xu et al., 2021).

This study aimed to assess the physical, chemical, and bioactive characterization of bitter orange (*C. aurantium*) samples at three stages of maturation. Additionally, it sought to compare these findings with the antioxidant potential of pasteurized pear orange (*C. sinensis*) juice.

2 MATERIALS AND METHODS

2.1 Samples

Bitter oranges (*C. aurantium*) were harvested in Maracaju, Mato Grosso do Sul, Brazil. Undamaged fruits were selected and categorized into three maturation stages: green, intermediate, and ripe. The fruits were then grouped accordingly. After selection, they were washed and sanitized with a 0.8% chlorine solution before being stored under refrigeration. Commercial pasteurized orange juice, produced from pear oranges (*C. sinensis*), was purchased from a local supermarket in Dourados, Mato Grosso do Sul, Brazil. Bitter orange juice was obtained by manual squeezing, while commercial orange juice was used directly as purchased.

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2.2 Water activity and pH

The water activity (a_W) of the samples was measured in triplicate using a hygrometer (Addium Inc., Aqualab model CX-2, São José dos Campos, SP, Brazil) at 25°C, with 1 mL of each sample. The pH of the samples was determined in triplicate using a digital pH meter (Digimed, model DM2, São Paulo, SP, Brazil) following the methodology of Spitzer and Werner (2002).

2.3 Total titratable acidity

The total titratable acidity (TTA) was determined through chemical titration of 5 mL of each sample. Phenolphthalein was used as the endpoint indicator, and the reaction was titrated with 0.1 N sodium hydroxide until a pink color appeared. TTA was expressed as g per 100 mL of juice. All experiments were conducted in triplicate, following the protocol described by IAL (2008).

2.4 Total soluble solids

The total soluble solids (TSS) of the samples were determined in triplicate using a refractometer device equipped with a 0–100 scale, and the results were expressed in °Brix (IAL, 2008).

2.5 Maturation index

The maturation index was obtained by the quotient of the values of TSS and TTA (IAL, 2008).

2.6 Vitamin C content

The oxidation–reduction titration method using iodine (iodimetry) was utilized to determine vitamin C content. To perform the procedure, 20 mL of the sample, 90 mL of 2% oxalic acid, and 5 mL of 1% starch solution were mixed and titrated with 0.1 N iodine solution. All experiments were carried out in triplicate (IAL, 2008).

2.7 Total phenolic content

The total phenolic (TP) content was determined in triplicate using the Folin–Ciocalteu method, with gallic acid as the standard. To prevent compound degradation, test tubes were wrapped in aluminum foil to shield them from light. Each sample was filtered and diluted with 0.5 mL of water, followed by the addition of 2.5 mL of 10% Folin–Ciocalteu reagent. The mixture was incubated at 25°C for 6 min before adding 2 mL of 7.5% sodium carbonate solution and adjusting the final volume to 8 mL with distilled water. The tubes were then placed in a water bath at 50°C for 15 min. After cooling, the absorbance was measured at 760 nm using a spectrophotometer. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation in terms of TP content (mg gallic acid equivalents [mg GAEs] per liter) (Fattahi et al., 2014).

2.8 Total flavonoid content

The total flavonoid (TF) content was measured in triplicate using the aluminum chloride method, with quercetin as the standard. To prepare the samples, 0.1 mL of juice and 4 mL of distilled water were added to a 10-mL volumetric flask. Next, 0.3 mL of 5% sodium nitrite was added, followed by 0.3 mL of 10% aluminum chloride after 5 min. The mixture was incubated at 25°C for 6 min, and then 1 mL of 1 M sodium hydroxide was added. The final volume was adjusted to 10 mL with distilled water and mixed thoroughly. The absorbance of the samples was measured at 510 nm using a spectrophotometer, with a blank as the reference. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation in terms of TF content (mg quercetin equivalents [mg QEs] per liter) (Fattahi et al., 2014).

2.9 Antioxidant activity

The antioxidant activity of the samples was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Orange juice samples were mixed with 95% methanol and 90 µM DPPH, resulting in final concentrations ranging from 0.01 to 2.00 mg mL^{-1}. The mixture was incubated at 25°C for 60 min, and the absorbance was measured at 515 nm using a spectrophotometer. Methanol was used as the baseline to set zero absorbance. The concentration of $DPPH^+$ in the reaction medium was calculated using a calibration curve generated by linear regression. The results were expressed as mean ± standard deviation. The percentage of the remaining $DPPH^+$ was plotted against the extract concentration to determine the effective concentration required to reduce the initial $DPPH^+$ concentration by 50% (EC_{50}). All experiments were conducted in triplicate (Espín et al., 2000).

2.10 Statistical analysis

The results of the determinations were submitted to Sisvar software for analysis of variance (ANOVA). The Tukey test was used to determine the significant differences between the samples, at 5% significance. The evaluations were performed with data obtained in triplicates, and the results were presented in mean ± standard deviation.

3 RESULTS AND DISCUSSION

The results for water activity, pH, TTA, TSS, and maturation index are presented in Table 1. The results for vitamin C content, TP content, TF content, and antioxidant activity (EC_{50}) are summarized in Table 2.

3.1 Water activity

Water activity (a_W) showed no statistically significant differences ($p \geq 0.05$) across the different maturation stages of the bitter orange. However, the pear orange juice exhibited a statistically significant difference ($p \leq 0.05$) compared to the other samples. The bitter orange juice had an a_W value of 0.981, aligning with the literature, which reports an average a_W of 0.988 for orange juices (Schmidt & Fontana Jr., 2020). The lower a_W value of 0.975 observed in the pasteurized orange juice is likely due to the heat treatment, which reduces the amount of free water in the juice.

Table 1. pH, water activity (a_w), total titratable acidity (TTA), total soluble solids (TSS), and maturation index in the samples of sour orange (*Citrus aurantium*) at different ripening stages and in the samples of pasteurized orange (*Citrus sinensis*) juice.

Sample	a_w	pH	Total titratable acidity (g 100 mL^{-1})	Total soluble solids (°Brix)	Maturation index (TSS/TTA)
Green bitter orange juice	$0.984^{a} \pm 0.000$	$2.31^{d} \pm 0.01$	$6.23^{a} \pm 1.08$	$10.93^{b} \pm 0.12$	$1.75^{b} \pm 0.52$
Intermediate bitter orange juice	$0.985^{a} \pm 0.000$	$2.56^{c} \pm 0.01$	$5.57^{a} \pm 0.24$	$8.50^{d} \pm 0.00$	$1.52^{b} \pm 0.21$
Ripe bitter orange juice	$0.981^{a} \pm 0.000$	$2.64^{b} \pm 0.01$	$6.19^{a} \pm 0.39$	$12.20^{a} \pm 0.17$	$1.97^{b} \pm 0.25$
Commercial sweet orange juice	$0.975^{b} \pm 0.000$	$3.69^{a} \pm 0.01$	$1.01^{b} \pm 0.07$	$10.47^{c} \pm 0.06$	$10.36^{a} \pm 0.05$

The evaluations are performed with data obtained in triplicates, and the results are presented as mean ± standard deviation. Means with the same lowercase letter in the same line do not differ statistically significantly at 5% ($p \geq 0.05$).

Table 2. Vitamin C content, total phenolic content, total flavonoid content, and antioxidant activity (EC_{50}) in samples of sour orange (*Citrus aurantium*) at different ripening stages and in samples of pasteurized orange (*Citrus sinensis*) juice.

Sample	Vitamin C content (mg 100 mL^{-1})	Total phenolic content (mg GAE L^{-1})	Total flavonoid content (mg QE L^{-1})	EC_{50} (mL L^{-1} of the sample)
Green bitter orange juice	$77.747^{a} \pm 4.485$	$842^{b} \pm 0$	$310.63^{c} \pm 7.65$	$280^{b} \pm 9$
Intermediate bitter orange juice	$72.193^{a} \pm 2.447$	$523^{d} \pm 9$	$374.38^{b} \pm 16.94$	$185^{c} \pm 8$
Ripe bitter orange juice	$58.310^{a} \pm 2.976$	$767^{c} \pm 11$	$116.09^{d} \pm 0.00$	$280^{b} \pm 10$
Commercial sweet orange juice	$79.135^{a} \pm 4.893$	$1{,}248^{a} \pm 18$	$442.87^{a} \pm 6.08$	$418^{a} \pm 7$

The evaluations are performed with data obtained in triplicates, and the results are presented as mean ± standard deviation. Means with the same lowercase letter in the same line do not differ statistically significantly at 5% ($p \geq 0.05$).

3.2 pH

Statistically significant differences ($p < 0.05$) were observed between all samples for pH, with pasteurized orange juice showing the highest value (3.69) (Table 1). The pH of 3.69 for the pasteurized pear orange juice is consistent with values reported in the literature for commercial pasteurized sweet orange juices, such as 3.78 ± 0.23 (Sugai et al., 2002), 3.52 (Danieli et al., 2009), 3.86–3.79 (Alegre & Sylos, 2015), 3.68–3.74 (Wibowo et al., 2015), 3.39–3.55 (Souza et al., 2017), and 3.6–3.8 (Souza et al., 2018).

In contrast, the pH values of bitter orange juice were statistically significantly lower ($p < 0.05$), ranging from 2.31 for green oranges to 2.64 for ripe oranges, which aligns with previous findings (e.g., 2.16 for bitter orange juice; Karadeniz, 2004). The lower pH values are attributed to the higher concentration of strong acids, which increases the H^+ ion concentration and, consequently, the acidity of the juice.

3.3 Total titratable acidity

The TTA did not differ statistically significantly ($p \geq 0.05$) between the maturation stages of bitter oranges. However, bitter orange juice had statistically significantly higher TTA ($p \leq 0.05$) compared to pear orange juice. The much lower TTA value observed for pear orange juice (1.01 g 100 mL^{-1}) is primarily due to the lower acid content of this variety rather than its maturation stage (Table 1).

Literature values for TTA (g 100 mL^{-1}) of pasteurized pear orange juice include 0.63 ± 0.14 (Sugai et al., 2002), 0.86 (Danieli et al., 2009), 0.68–0.70 (Alegre & Sylos, 2015), 0.77–0.82 (Wibowo et al., 2015), 0.39–0.41 (Souza et al., 2017), and 0.82–0.96 (Souza et al., 2018). In contrast, bitter orange juice has reported values as high as 4.848 g 100 mL^{-1} (Karadeniz, 2004). Previous studies have noted that bitter oranges can exhibit acidity levels up to 45 times higher than sweet oranges (Moufida & Marzouk, 2003). Notably, Brazilian legislation does not specify minimum or maximum acidity limit for orange juices (Brasil, 2018).

3.4 Total soluble solids

All samples showed statistically significant differences in TSS values ($p \leq 0.05$). The ripe bitter orange juice exhibited the highest TSS value (12.20 °Brix), while the intermediate stage had the lowest value (8.50 °Brix) (Table 1). Typically, TSS increases as oranges ripen due to higher sugar content, which is crucial for juice quality.

The TSS value for pasteurized pear orange juice complies with Brazilian legislation, which requires a minimum of 10 °Brix at 20°C for pear orange juices (Brasil, 2018). Literature values for TSS (°Brix) in pasteurized pear orange juice include 11.39 ± 1.09 (Sugai et al., 2002), 13.0 (Danieli et al., 2009), 10.99–12.00 (Alegre & Sylos, 2015), 11.01 (Wibowo et al., 2015), 10.8–11.6 (Souza et al., 2017), and 12.25–13.00 (Souza et al., 2018). For bitter orange juice, TSS values of 10.0 ± 0.3 have been reported (Karadeniz, 2004).

3.5 Maturation index

The maturation index (TSS/TTA) did not present any statistically significant differences ($p > 0.05$) for bitter orange juices (Table 1). However, the values obtained for the bitter orange juice at different maturation stages were much below the value of 7.0, indicated as the minimum for orange juices by the Brazilian legislation (Brasil, 2018). This was expected due to the high acidity of these juices (Moufida & Marzouk, 2003). The maturation index represents the balance between the content of sugars and organic acids in the fruit and is associated with the juice's taste. It is an important parameter to be considered when adjusting the harvesting time and the distance to the destination (Domingues et al., 2021).

3.6 Vitamin C content

A statistically significant decrease ($p < 0.05$) in vitamin C content was observed in bitter orange juice as maturation progressed. However, no statistically significant difference ($p \geq 0.05$) was found between the vitamin C content of ripe bitter orange juice and commercial pasteurized pear orange juice (Table 2).

Literature reports varying vitamin C levels depending on the orange variety. The Brazilian Food Composition Table (TACO, 2011) lists 73.3 mg 100 g^{-1} for pear oranges and 44.3 mg 100 g^{-1} for bitter oranges. Other studies report the vitamin C content (mg 100 mL^{-1}) for pasteurized pear orange juice as follows: 32.36 (Danieli et al., 2009), 32.479–46.485 (Alegre & Sylos, 2015), 47.0 (Wibowo et al., 2015), 46.10–86.37 (Souza et al., 2017), and 32.0–36.0 (Souza et al., 2018). Comparatively, our results (Table 2) indicate that both bitter and pear orange juices generally have higher vitamin C content than these references, regardless of the maturation stage.

3.7 Total phenolic content

All samples exhibited statistically significant differences in TP content ($p < 0.05$). The commercial pasteurized pear orange juice had the highest TP value (1,248 mg GAE L^{-1}), while intermediate bitter orange juice recorded the lowest value (523 mg GAE L^{-1}) (Table 2).

Reported literature values for TP (mg GAE L^{-1}) in pasteurized pear orange juice include 667.4–715.4 (Alegre & Sylos, 2015). For bitter orange juice, a TP value of 823.13 ± 17.18 mg GAE L^{-1} has been documented (Karoui & Marzouk, 2013). TP levels in bitter oranges are typically higher in the peel than in the juice (Ersus & Cam, 2007; Wen et al., 2021). Furthermore, the antioxidant activity varies with the ripening stage (Moulehi et al., 2012).

3.8 Total flavonoid content

The highest TF content was observed in commercial pasteurized pear orange juice (442.87 mg QE L^{-1}), followed by intermediate bitter orange juice (374.38 mg QE L^{-1}). All treatments differed statistically significantly from each other ($p < 0.05$) (Table 2).

Literature values for TF content in pasteurized orange juice include 320.06 ± 5.42 and 328.04 ± 5.10 mg L^{-1} for lane late orange juice treated under different conditions (Stinco et al., 2020) and 136.91 ± 0.17 mg L^{-1} for bitter orange juice (Karoui & Marzouk, 2013). Despite undergoing thermal processing, pasteurized juice exhibited the highest flavonoid values. Studies indicate that flavonoids are generally stable during heat treatment (Gil et al., 2002; Lu et al., 2018) due to their chemical structure and extraction methods (Biesaga, 2011). Even in cases of thermal degradation, the resulting products may retain or enhance the antioxidant activity (Chaaban et al., 2017).

3.9 Antioxidant activity

The EC_{50} value represents the amount of antioxidants required to reduce the radical concentration by 50%; thus, lower EC_{50} values indicate higher antioxidant capacity (Singh et al., 2020).

In Table 2, green and ripe bitter orange juices showed similar antioxidant activity (EC_{50} = 280 mL L^{-1}, $p \geq 0.05$), while intermediate bitter orange juice demonstrated the lowest EC_{50} value (185 mL L^{-1}), indicating the highest antioxidant activity among the samples. These results suggest that both green and ripe bitter oranges have a higher antioxidant capacity than intermediate-stage fruits.

Although the antioxidant capacity generally increases with fruit ripening, surface color alone may not be the best indicator of ripeness or antioxidant properties (Andrade et al., 2002). In contrast, commercial pasteurized pear orange juice exhibited a significantly higher EC_{50} value (418 mL L^{-1}), reflecting lower antioxidant activity.

Reported EC_{50} values include 267.49 g extract per liter of methanolic fraction for fresh pear orange juice (Duzzioni et al., 2009), comparable to the antioxidant activity observed in this study. However, pasteurization may reduce the antioxidant activity.

4 CONCLUSION

The comparative analysis of bitter and pear orange juices revealed some differences in their properties and bioactive compound contents, influenced by fruit variety, maturation stage, and processing methods. Vitamin C content decreased with the maturation of bitter oranges, yet the ripe bitter orange juice exhibited levels comparable to commercial pasteurized pear orange juice. TP and TF contents were highest in pasteurized pear orange juice, highlighting the stability of these compounds during thermal processing. Notably, bitter orange juices demonstrated greater variability in antioxidant activity (EC_{50}) across maturation stages, with intermediate-stage fruits showing the strongest capacity. However, in comparison to the pasteurized pear orange juice, the degree of maturation was not a determining factor for antioxidant activity. These findings emphasize the unique nutritional and functional profiles of orange juice varieties. Bitter oranges, despite being less commercially prevalent, offer distinct advantages in bioactive compound richness and antioxidant activity. Future research should explore further correlations between chemical and physical parameters with maturation stages and processing techniques, to maximize the nutritional benefits of bitter orange juice.

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