Stability and antioxidant activity of bioactive compounds in Cerrado fruit juices

during storage

Estabilidade e atividade antioxidante de compostos bioativos em sucos de frutas do Cerrado

durante o armazenamento

Estabilidad y actividad antioxidante de compuestos bioactivos en jugos de frutas del Cerrado

durante el almacenamiento

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Nathane Silva Resende ORCID: https://orcid.org/0000-0002-3311-8182 Faculty Única, Brazil E-mail: nathane.resende@hotmail.com Vanessa Rios de Souza ORCID: https://orcid.org/0000-0003-1377-821X Federal University of Lavras, Brazil E-mail: vanessardsouza@gmail.com Elisângela Elena Nunes Carvalho ORCID: https://orcid.org/0000-0002-1124-8066 Federal University of Lavras, Brazil E-mail: elisangelacarvalho@ufla.br João Renato de Jesus Junqueira ORCID: https://orcid.org/0000-0001-8552-7087 Federal University of Mato Grosso do Sul, Brazil E-mail: vanessardsouza@gmail.com Eduardo Valério de Barros Vilas Boas ORCID: https://orcid.org/0000-0002-0252-695X Federal University of Lavras, Brazil E-mail: evbvboas@ufla.br

Abstract

The development of beverages with differential characteristics is mandatory, due to changes in the food habits of the population. The production of ready-to-drink fruit juices presents an interesting process for the shelf life extension, integral utilization of the fruits and the development of new products. The introduction of Cerrado fruit in this segment increases the visibility of these native species that are still unexploited. This study aimed to evaluate the bioactive compound stability of juices (marolo, cagaita and mixed: marolo + cagaita) during the refrigerated storage. The content of the vitamin C, total phenolics and antioxidant activity (auto-oxidation of the β -carotene/linoleic acid system, ABTS •+ and DPPH• free scavenging radical) in the juices were determined during the storage. The use of marolo and cagaita in the development of ready-to-drink beverages was suitable. A reduction in the vitamin C, total phenolics and antioxidant activity (evaluated by three methodologies) was observed during the 90 days. Considerable bioactive retention was obtained, highlighting the importance of the Cerrado fruit juices production. **Keywords:** Annona crassiflora Mart.; Eugenia dysenterica; Vitamin C; Antioxidant activity.

Resumo

Em função das alterações nos hábitos alimentares da população, o desenvolvimento de bebidas com características diferenciadas se torna interessante. O processo de obtenção de sucos "prontos para consumo" auxilia na extensão da vida-útil dos frutos, além de aproveitamento integral e desenvolvimento de novos produtos. A introdução de frutos do Cerrado neste segmento aumenta a visibilidade destas espécies nativas, que ainda são poucas exploradas. Este trabalho teve como objetivo avaliar a estabilidade dos compostos bioativos de sucos (marolo, cagaita e misto: marolo + cagaita) durante o armazenamento refrigerado. O teor de vitamina C, fenólicos totais e atividade antioxidante (auto-oxidação do sistema β -caroteno/ácido linoleico, radicais livres ABTS + e DPPH•) nos sucos foram determinados durante o armazenamento. A utilização do marolo e da cagaita no desenvolvimento de bebidas prontas foi adequado. Uma redução na vitamina C, fenólicos totais e atividade antioxidante (avaliada por três metodologias) foi observada durante os 90 dias. Observou-se considerável retenção de compostos bioativos, destacando as potencialidades do emprego destes frutos do Cerrado na elaboração de sucos.

Palavras-chave: Annona crassiflora Mart.; Eugenia dysenterica; Vitamina C; Atividade antioxidante.

Resumen

El desarrollo de bebidas con características diferenciales es obligatorio, debido a los cambios en los hábitos alimentarios de la población. La producción de jugos de frutas listos para beber presenta un proceso interesante para la extensión de la vida útil, el aprovechamiento integral de las frutas y el desarrollo de nuevos productos. La introducción de la fruta del Cerrado en este segmento aumenta la visibilidad de estas especies nativas que aún no se explotan. Este estudio tuvo como objetivo evaluar la estabilidad de los compuestos bioactivos de jugos (marolo, cagaita y mixto: marolo + cagaita) durante el almacenamiento refrigerado. El contenido de vitamina C, fenoles totales y actividad antioxidante (autooxidación del sistema β -caroteno/ácido linoleico, ABTS + y DPPH• captación de bebidas listas para beber resultó adecuado. Se observó una reducción en la vitamina C, fenoles totales y actividad antioxidante (evaluada por tres metodologías) durante los 90 días. Se obtuvo una retención bioactiva considerable, destacando la importancia de la producción de jugos de frutas del Cerrado.

Palabras clave: Annona crassiflora Mart.; Eugenia dysenterica; Vitamina C; Actividad antioxidante.

1. Introduction

The demand for natural and practical foods has increased in recent years, mainly because of the nutritional and functional benefits of their consumption, coupled to the sensorial characteristics and sustainable development. The beverage technologies, including ready-to-drink juices may support this request (Azzurra et al., 2019; Noguera et al., 2021).

Brazil presents a great species native diversity, and techniques which improve the valorization of their biomes (such as the Cerrado) are necessary. Presenting potential for economic prominence, most of the native Cerrado fruits still have limited commercialization, despite social identity significance and environmental relevance (Bortolotto et al., 2021).

In general, Cerrado fruits present nutritional potential, as sources of natural vitamins, minerals, fiber and bioactive compounds. Due to seasonality and perishability, techniques which extend the shelf life, with properties maintenance, and the obtention of differentiated value-added products are required (Fank-de-Carvalho et al., 2015; Schiassi et al., 2018).

The marolo fruit (*Annona crassiflora* Mart.) presents exotic characteristics, with pleasant flavor and aroma, besides high antioxidant potential, due to the presence of lycopene, carotenes, tocopherols and vitamins (Arruda et al., 2017; Morais Cardoso et al., 2013). The cagaita (*Eugenia dysenterica*) presents peculiar flavor, with sensorial attributes appreciated by children. Its consumption is restricted to the Cerrado microregion, in the form of jellies and jams (Arruda et al., 2015).

The functional properties of these fruits boost their utilization. The adequate ingestion of bioactive compounds are related to health benefits, reducing the risk of chronic diseases development, by limiting the reactive oxygen species (ROS) production (Mirmiran, 2014).

Beyond the phenolic compounds, vitamin C presents numerous benefits associated with its consumption, as: collagen and neurotransmitters synthesis, facilitates mineral absorption, and promotes resistance to infections, acting in healing processes (Mendonça et al., 2021; Rinaldi et al., 2021). Côrrea Neto & Faria (1999) stated that the degradation of vitamin C may be associated with the juice pH. The values range from 4.0 to 6.0, so it is important to study the concentration and stability of vitamin C in different fruit juice formulations.

Since the consumption of cagaita and marolo are restricted, a practical alternative for their utilization would be a ready-to-drink preparation. As the bioactive compounds stability reduces with storage time, it is important to evaluate their levels during this period. Thus, the objective of this study was to evaluate the stability of bioactive compounds (vitamin C and total phenolics) and antioxidant activity of Cerrado fruit juices during refrigerated storage.

2. Methodology

Statistical design

The experiment was carried out in a completely randomized design (CRD), in a 7×3 factorial scheme, with 7 storage times (0, 15, 30, 45, 60, 75, and 90 days) and 3 treatments (three juice formulations) with three replications, totaling 63

samples $(7 \times 3 \times 3)$, the experimental plot consisted of 100 mL of juice (a glass bottle).

Juice preparation

The marolo and cagaita pulps were obtained from were obtained from Sitio do Belo – Frutas nativas® processing industry (Piraibuna, São Paulo, Brazil). Pulp received no water addition nor heat treatment. Prior to the experiments, the pulps were stored at -18 ± 1 °C. The different treatments are presented in Table 1. Such formulations were obtained according to Schiassi et al. (2018).

Formulation	Marolo pulp [v/v]	Cagaita pulp [v/v]	Water [v/v]	Sucralose [w/v]
Marolo juice	40	0	60	0.02
Cagaita juice	0	50	50	0.02
Mixed juice	20	30	50	0.02

Table	1	- Juice	formu	lations
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Source: Authors.

The prepared juices were packed in amber glass bottles (100 mL) and pasteurized (90 s / 80 °C) in a shaken water bath (Quimis®, Q215M model, Diadema, Brazil). After that, the bottles were cooled in ice water and stored at $5^{\circ} \pm 1$ °C for 90 days. The following analyses were performed at 0, 15, 30, 45, 60, 75 and 90 days.

Bioactive compounds

Vitamin C (ascorbic acid) content was determined by the colorimetric method using 2,4-dinitrophenyl hydrazine (Strohecker & Henning, 1967). Ascorbic acid was extracted with 0.5% oxalic acid, filtered, and dosed in the extract, using ascorbic acid as a standard. The data were acquired at 520 nm on a spectrophotometer. The assay was carried out in triplicate, and the results were expressed as mg of ascorbic acid per 100 mL of sample.

The total phenolic content (TPC) was determined based on the Folin-Ciocalteu methodology with the adaptations (Waterhouse, 2002). For this, 5 mL of the juice was mixed with 20 mL of methanol (50% v/v). The resulting solution was incubated under light protection for 60 min, and then centrifugated at 14000 rpm for 15 min.

The supernatant was separated from the residue. Subsequently, approximately 20 mL of acetone (70% v/v) was mixed into the residue and the process described above was repeated. The extract was conducted in triplicate, and the result was expressed in gallic acid (mg gallic acid equivalent/100 mL sample).

Antioxidant activity (AA)

The antioxidant activity (AA) was determined using three methodologies: auto-oxidation of the β -carotene/linoleic acid system (Rufino et al., 2006), ABTS ++ (Rufino et al., 2007a) and DPPH• free scavenging radical (Rufino et al., 2007b). All the analyses were performed in triplicate.

For the β -carotene/linoleic acid system, the extract was measured using UV-Visible spectrophotometer at 470 nm, and the results were expressed according to the Eq. 1:

% protection =
$$100 - \left[\frac{\text{Sample Reduction Abs}}{\text{System Reduction Abs}} \times 100\right]$$
 (1)

The capture of the 2,20'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS \bullet +) radical was measured using UV-Visible spectrophotometer at 734 nm, and the results were expressed as μ M trolox/g.

The DPPH• scavenging ability of the antioxidant activity was measured using UV-Visible spectrophotometer at 515 nm, and the results were expressed in EC50 (mL sample/ g DPPH), which represents the amount of sample necessary to reduce the radicals by the half.

Statistical analyses

All experiments were conducted in triplicates. Data were subjected to analysis of variance (ANOVA) using software SISVAR[®] (Ferreira, 2011). Scot Knott's test was used to compare means at the 5% significance level (p < 0.05).

3. Results and Discussion

The vitamin C content during the refrigerated storage is presented in Figure 1.



Figure 1 - Mean values of vitamin C content during storage of fruit juice from the Brazilian Cerrado.

Same letters at the same storage time, do not differ significantly according to Scott Knott's test (p < 0.05). Source: Authors.

The aim of the study was to verify the stability of vitamin C, so the graphs focused on expressing these differences at each time point for each sample (one-way analysis). However, throughout the results, it is also possible to note analysis in relation to time, as the result of degradation in all juices was expected, we chose to present the percentage of differences.

According to Figure 1, at t = 0 (after the juice processing), the mixed juice presented a lower vitamin C content, when compared to the other formulations (statistical difference (p > 0.05). The cagaita and marolo juices presented, at t = 0, 26.12 and 25.96 mg of vitamin C, respectively, while the mixed juice presented 24.87 mg of vitamin C. This fact may be related to the different isolated juice concentrations, which influences the vitamin C content. However, throughout the experiment, the vitamin C total reduction (%) in this treatment (marolo + cagaita juice) was 31%, considering the 90 days.

Vitamin C content in marolo juice was reduced by 36%, while cagaita juice reduced its initial content by 38%. Despite different percentages with regard to total reduction, at the end of the experiment there was no significant difference (p < 0.05), as presented by the Figure 1.

During the refrigerated storage, a linear decrease in the vitamin C was observed for the marolo and cagaita juices, while quadratic adjustment performed best for the mixed juice (Figure 1). The highest vitamin degradation was found in the cagaita juice, which presented lower values after 60 days. At 90 days, no statistical difference (p > 0.05) was observed for the different treatments.

The vitamin C degradation is related to the temperature and storage conditions, since this compound is heat- light- and oxygen sensible. The refrigerated temperature and amber glass assisted the vitamin C preservation. However, the oxygen

presence leads to the oxidation reactions, promoting the dehydroascorbic acid development. In addition, at lower pH values, the dehydroascorbic acid undergoes irreversible rearrangement, reducing its bioavailability (Leão-Araújo et al., 2019).

Chim et al. (2013) evaluated the temperature effect on the vitamin C content in acerola nectar and observed that this parameter was critical. Higher vitamin C retention was performed at 8°C, comparing with 25 °C. Similar findings were reported by Thakur et al. (2017) and Thakur et al. (2018) during the storage of a *Myrica nagi* (Box myrtle) drink and a ready-to-serve aonla fruit juice based beverage, respectively.

A gradual decrease in the TPC was observed during storage (Figure 2). A reduction in the TPC was observed for all the treatments, linearly for marolo and cagaita isolated juices, and quadratically for the mixed juice.

At initial (t = 0) and final (t = 90 days) periods, significant differences ($p \le 0.05$) were observed for the treatments. The mixed fruit juice presented the highest TPC during the entire storage period (Figure 2).

The TPC reduction can be attributed to residual enzymatic activity during storage (Marszałek et al., 2018). In spite of the pasteurization process, remaining enzymes (polyphenol oxidase - PPO and peroxidase - POD) are active, and these proteins are responsible for the TPC degradation (Paciulli et al., 2016).

Figure 2 - Mean values of total phenolic content during storage of fruit juice from the Brazilian Cerrado.



Same letters at the same storage time, do not differ significantly according to Scott Knott's test (p<0.05). Source: Authors.

Marszałek et al. (2018) evaluated the profile changes of apple juice under supercritical carbon dioxide and long-term cold storage and observed a reduction in the TPC. Corleto et al. (2018) studied the beetroot juice stability, and observed that at 25 °C, significant decrease in TPC occurred, but at lower temperatures, the levels remained relatively stable.

Vitamin C and phenolic compounds possess strong AA, which are positively correlated with the antioxidant analyses (Figs. 3, 4 and 5).



Figure 3 - Antioxidant activity of fruit juice from the Brazilian Cerrado evaluated by β -carotene bleaching assay.

Same letters at the same storage time, do not differ significantly according to Scott Knott's test (p<0.05). Source: Authors.

For evaluating the AA of vegetable products, several methods have been applied. These techniques can be based on the organic radical capture (such as ABTS++ and DPPH+ methods) and on the quantification of formed products during the lipid peroxidation (β -carotene method). Since various reaction mechanisms are related, more than one methodology is indicated for quantifying all the antioxidant substances (Schiassi et al., 2018).

A linear reduction of β -carotene protection percentage was observed for all the treatments (Figure 3). This biological substance is an oxidable substrate, against hydroperoxide-derived free radicals produced by linoleic acid oxidation. Such a result is related to the natural bioactive compounds degradation, neutralizing the linoleate-free radical and other free radicals (Rajamani et al., 2018).





Same letters at the same storage time, do not differ significantly according to Scott Knott's test (p<0.05). Source: Authors.



Figure 5 - Antioxidant activity of fruit juice from the Brazilian Cerrado determined by the DPPH method.

Same letters at the same storage time, do not differ significantly according to Scott Knott's test (p<0.05). Source: Authors.

No statistical difference (p > 0.05) was observed up to the 15th day for all the treatments (Figure 3). Lower ability to protect β -carotene against free radicals was observed for the mixed fruit juice. The marolo juice presented higher TPC (Figure 2), as well as higher ability to protect β -carotene from oxidation (Figure 3).

According to the classification proposed by Hassimotto et al. (2005), fruits are classified into high (> 70% inhibition), intermediate (40-70% inhibition) and low antioxidant capacity (<40% inhibition). In this classification, the juices were classified as intermediate antioxidant capacity up to the 75th day (Figure 3). At t = 90, the cagaita and the mixed juices were classified as low antioxidant capacity.

Tembo et al. (2017) related high vitamin C, TPC and AA, when evaluating the thermal treatment and storage of baobab fruit (*Adansonia digitata*) pulp. Ibrahima et al. (2013) associated the antioxidant activity reduction to the vitamin C decreasing, similar to the results observed in the present study.

The AA is related to the chemical composition of the juice and the degradation of the compounds over the storage. Kim et al. (2018) observed the vitamin C degradation during the different fruit storage. This vitamin eliminates free radicals, by reacting with ROS, releasing as a semi-dehydroascorbic acid product, which demonstrates its direct relationship with the antioxidant activity (Xie et al., 2021).

Evaluating the β -carotene/linoleic acid system for lipid oxidation inhibition, Zou et al. (2016) observed a positive relationship between the antioxidant capacity of citrus fruits and the TPC. Margraf et al. (2016) studied purple juice and concluded that higher TPC leads to a higher lipid oxidation inhibition.

A decrease in the AA during the storage was also observed by the ABTS++ methodology (Figure 4). A quadratic model was fitted to the experimental data for all the treatments. Significant differences ($p \le 0.05$) were observed, and the marolo juice presented higher AA than cagaita and the mix juices. Schiassi et al. (2018) observed that the marolo fruit presents higher AA than the cagaita fruit, in accordance with the obtained results for their juices.

The DPPH• (EC50) results are presented in the Figure 5. There was an increase in the EC50 values for all the treatments. The suitability of quadratic behavior was observed for the marolo juice, whereas the cagaita and mixed juices adjusted to linear models. The higher EC50, the lower the AA (Qian et al., 2021), therefore, the reported increase reflects a reduction in the antioxidant capacity during the storage period. In general, marolo juice presented the highest AA, similar to the results shown in Figs. 3 and 4.

The flavonoids solubility may be associated with their respective reaction media, and this fact could influence the results presented by the DPPH• and ABTS•+ methods. Nevertheless, DPPH• and ABTS•+ radical scavenging activities are

commonly used to measure natural antioxidant activities, due to the stability of nitrogen radicals and the easily result detection (Olszowy & Dawidowicz, 2018).

As a general remark, even with the reduction in the vitamin C content and TPC (and consequently in AA), the Cerrado fruit juices are interesting for processing, for providing different products and economic, social, and environmental development to this region.

4. Conclusion

The suitability of Cerrado fruit juice (in terms of bioactive compounds and antioxidant activity) was achieved. The use of marolo and cagaita in the development of ready-to-drink beverages was evaluated, and the refrigerated storage was carried out. A reduction in the vitamin C, TPC and AA (evaluated by three methodologies) was observed during the 90 days, but its values demonstrated the importance of the use of this native and unexploited fruits in the juice elaboration. Nevertheless, further analyzes are needed to evaluate the nutritional, sensory and microbiological characteristics of the juices, and their stability at different temperatures.

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