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Bioactive compounds and antioxidant activity of cassava roots

Compuestos bioactivos y actividad antioxidante de raíces de yuca

Compostos bioativos e atividade antioxidante de raízes da mandioca

Andressa, Sousa Pereira[1]; Amanda, Sousa Pereira[1]; Douglas, Martins da Costa[1]; Caroline Roberta, Freitas Pires[1]; Diego, Neves de Sousa[2]; Gustavo, Azevedo Campos[2]

1- Universidade Federal do Tocantins. Brasil.
2- Embrapa Pesca e Aquicultura. Brasil.

*E-mail: diegocoop@hotmail.com

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Abstract
The aim of the study was to evaluate the influence of storage time on the levels of bioactive compounds and antioxidant activity in frozen cassava roots from the cultivars BRS 400 (pink pulp), Cacau (yellow pulp) and Jari (white pulp). The roots were packed in polyethylene packaging, frozen at -20± 2°C and stored for 180 days. Samples were analyzed every 45 days of storage to determine total phenolics, lycopene, β-carotene, total carotenoids and antioxidant activity. Pink-fleshed roots exhibited the highest levels of phenolic compounds, lycopene, and antioxidant activity, while yellow-fleshed roots presented the highest β -carotene content. In contrast, white-fleshed roots presented the lowest levels of bioactive compounds and antioxidant activity. The cultivars BRS 400 and Cacau showed significant losses in bioactive compounds and antioxidant activity during storage, whereas Jari showed no significant changes in these variables, except for a decrease in lycopene. Additionally, pink- and yellow-fleshed roots exhibited a reduction in total carotenoid content over time. Overall, the white-fleshed cultivar had the lowest initial levels of bioactive compounds and antioxidant activity, as well as the smallest losses during storage.

Keywords: Nutritional quality; Freezing; Functional compounds.

Resumen
El objetivo del estudio fue evaluar la influencia del tiempo de almacenamiento en los niveles de compuestos bioactivos y actividad antioxidante en la yuca, de las variedades BRS 400 (pulpa rosada), Cacau (pulpa amarilla) y Jari (pulpa blanca), congelada. Las raíces fueron empacadas en envases de polietileno, congeladas a -20± 2°C y almacenadas durante 180 días. Las muestras fueron evaluadas cada 45 días de almacenamiento para los niveles de fenoles totales, licopeno, β-caroteno, carotenoides totales y actividad antioxidante. Las raíces de pulpa rosada se destacaron por los valores más altos de compuestos fenólicos, licopeno y actividad antioxidante, las raíces de pulpa amarilla por el mayor contenido de beta-caroteno, mientras que las raíces de pulpa blanca presentaron los niveles más bajos de compuestos bioactivos y menor actividad antioxidante. La yuca ‘BRS 400’ y ‘Cacau’ mostraron pérdidas significativas de los compuestos bioactivos y la actividad antioxidante evaluada, mientras que la yuca ‘Jari’ no mostró cambios en estas variables, excepto por la disminución observada en el licopeno, durante el almacenamiento. Las raíces rosadas y amarillas mostraron una reducción en el contenido de carotenoides totales durante el almacenamiento. La variedad de pulpa blanca presentó los niveles más bajos de compuestos bioactivos y actividad antioxidante, y la menor pérdida significativa de estos compuestos durante el almacenamiento.

Palabras clave: Calidad nutricional; Congelación; Compuestos funcionales.

Resumo
O trabalho teve como objetivo avaliar a influência do tempo de armazenamento sobre os teores de compostos bioativos e atividade antioxidante em mandiocas, das cultivares BRS 400 (polpa rosada), Cacau (polpa amarela) e Jari (polpa branca), congeladas**.** As raízes foram acondicionadas em embalagens de polietileno, submetidas ao congelamento a -20± 2°C e armazenadas por 180 dias. As amostras foram avaliadas a cada 45 dias de armazenamento quanto aos teores de fenólicos totais, licopeno, β-caroteno, carotenoides totais e atividade antioxidante. Raízes de polpa rosa se destacaram pelos valores mais elevados de compostos fenólicos, licopeno e atividade antioxidante, as de polpa amarela, pelo maior teor de betacaroteno, enquanto as de polpa branca apresentaram os menores teores de compostos bioativos e menor atividade antioxidante. Mandiocas ‘BRS 400’ e ‘Cacau’ apresentaram perdas significativas dos compostos bioativos e atividade antioxidante avaliados, enquanto a mandioca ‘Jari’ não apresentou alterações nessas variáveis, à exceção da queda observada no licopeno, ao

longo do armazenamento. As raízes de coloração rosa e amarela apresentaram redução no teor de carotenoides totais durante o armazenamento. A cultivar de polpa branca apresentou os menores teores de compostos bioativos e atividade antioxidante, e menor perda significativa desses compostos durante o armazenamento.

Palavras-chave: Qualidade nutricional, Congelamento; Compostos funcionais.

Introduction

Antioxidants are chemical compounds capable of preventing or reducing oxidative damage to lipids, proteins, and nucleic acids caused by reactive oxygen species. These species, generated in the body, are responsible for cellular damage and are associated with various physiological and pathological conditions, such as inflammation, cardiovascular diseases, cancer, and aging [1].

The consumption of plant-based foods has been associated with reduced mortality and morbidity from chronic diseases. This effect is largely attributed to the presence of antioxidant compounds in different parts of these foods. Among these compounds are ascorbic acid, carotenoids, and phenolic compounds, which occur in varying concentrations in vegetables [2].

Cassava roots have an average composition of 61.55% moisture, 0.87% ash, 0.65% protein, 0.13% lipids, and 1.25% fiber in their integral matter. In terms of micronutrients, they contain retinol, thiamine, riboflavin, niacin, and ascorbic acid, as well as mineral elements such as calcium, iron, magnesium, and zinc [3].

Cassava is one of the most widely produced and consumed food crops in developing countries and has diverse culinary applications [3]. Its consumption has increased, particularly due to the presence of carotenoids in the pulp, especially β-carotene and lycopene. In addition to imparting yellow or pink coloration—distinct from the traditional white—these compounds contribute to the functional value of cassava, making it an important dietary source of vitamin A and lycopene [4].

The availability of fresh cassava roots for direct consumption has declined due to their high perishability and limited visual appeal. As a result, demand for pre-cooked and frozen cassava products has increased, particularly in medium and large urban centers [5]. Freezing has been proposed as an effective alternative to extend shelf life and provide a more convenient product for consumers [6].

Freezing is considered an efficient preservation method because it slows physiological and microbiological deterioration. Lower storage temperatures reduce the rate of biochemical and microbial changes [3]. However, frozen storage can lead to a reduction in antioxidant compounds, thereby affecting the functional properties, sensory characteristics, and nutritional value of the product [2, 7].

Therefore, this study aimed to evaluate the influence of storage time on the concentration of bioactive compounds and antioxidant activity in frozen cassava roots from the cultivars BRS 400 (pink pulp), Cacau (yellow pulp), and Jari (white pulp).

Materials and Methods

In this study, cassava roots from the cultivar BRS 400 (pink pulp) were used, along with roots from the cultivars Cacau (yellow pulp) and Jari (white pulp). All cultivars were supplied by the São João Irrigated Fruit Farming Project, located in the rural area of Porto Nacional, Tocantins, and managed by the Brazilian Agricultural Research Corporation (Embrapa). The cultivation area is situated at an altitude of 220 m, with sandy latosol soil and basal fertilization applied in the planting furrow using simple superphosphate at a rate of 50 g per linear meter. Plant density was 10,000 plants per hectare, with a spacing of 1 m × 1 m, and micro-sprinkler irrigation was applied every two days.

Twelve months after planting, the roots were harvested and transported to the Food Technology Laboratory at the Federal University of Tocantins (UFT), Palmas campus. The roots were washed under running water and selected based on pulp color and the absence of visible deterioration. They were then immersed in a sodium hypochlorite solution (100 ppm) for 15 minutes.

Root processing followed the method described by [5] with modifications. Minimal processing included manual peeling, removal of the ends, washing under running water, cutting the central portion of the roots into 10 cm cylinders, and longitudinally sectioning these into four parts. The samples were then immersed for 10 minutes in a sanitizing solution containing 150 mg/L of active chlorine, rinsed for 5 minutes in a solution containing 5 mg/L of the same sanitizer, and drained for 5 minutes using a stainless-steel colander. The water temperature during washing, sanitization, and rinsing was maintained at 24 °C. The processing area and all utensils were previously sanitized, and the ambient temperature was maintained at 22 ± 2 °C. Personal protective equipment (PPE) was used throughout the procedure.

The minimally processed samples (200 g) were packaged in low-density polyethylene (LDPE) bags with a thickness of 120 µm, sealed using a commercial heat sealer, and stored at −20 ± 2 °C until analysis.

After minimal processing, analyses were performed on the day of harvest (time 0) and after

45, 90, 135, and 180 days of storage to quantify bioactive compounds and antioxidant activity.

Extracts for the determination of antioxidant activity and bioactive compounds (total phenolics, total carotenoids, β-carotene, and lycopene) were prepared according to the methodology described by Silva et al. (2014) [7]. Briefly, 20 g of root from each cultivar was weighed, homogenized, and mixed with 20 mL of acetone and 60 mL of hexane heated to 60 °C. The mixture was then transferred to a light-protected container and stored at 10 ± 2 °C for 24 h. After this period, the extracts were vacuum-filtered and used for subsequent analyses.

Total phenolic content was determined using the Folin–Ciocalteu method, as described by Waterhouse et al. (2002) [8]. Phenolic content was calculated from a gallic acid standard curve, and the results were expressed as mg of gallic acid equivalents per gram of sample (mg GAE/g extract). The quantification of total carotenoids, β-carotene, and lycopene was performed according to the method of Zscheile and Porter (1947) [9].

Antioxidant activity was determined following the method proposed by Rufino et al. (2010) [10], based on the scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH, 60 µM) radical, using the following equation:

$$\text{SRL\%} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}}\right] x100$$

A completely randomized design was adopted, with four replicates, arranged in a 3 × 5 factorial scheme corresponding to three cultivars (BRS 400, Cacau, and Jari) and five storage times (0, 45, 90, 135, and 180 days).

The results were subjected to analysis of variance (ANOVA) and regression analysis. Means were compared using Tukey's test at a 95% confidence level. Statistical analyses were performed using SISVAR software [11].

Results and Discussion

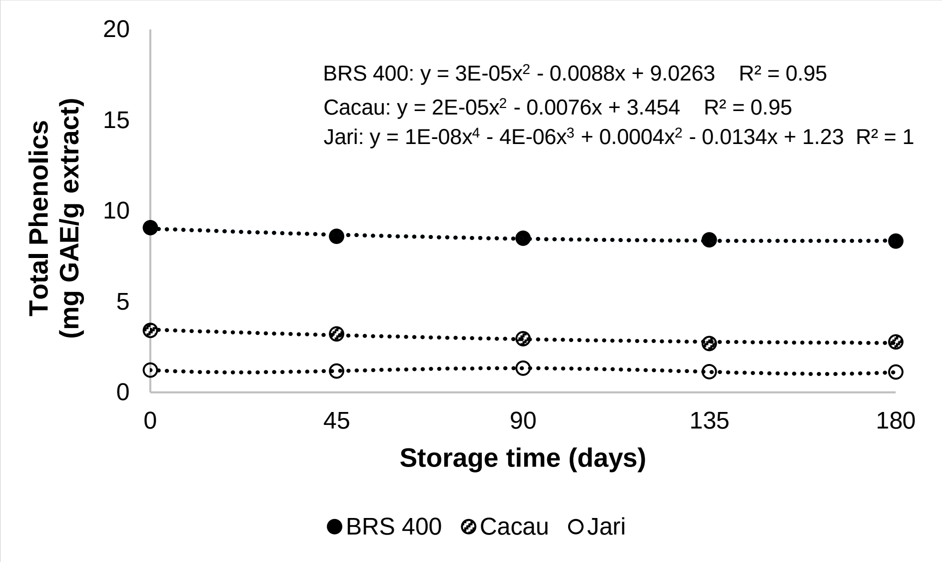
The effects of storage time on the bioactive compounds and antioxidant activity of cassava are presented in Table 1.

Table 1. Mean values of total phenolics, total carotenoids, lycopene, β-carotene, and antioxidant activity in cassava roots over the storage period. Palmas - TO, 2020(1).

Cultivars	**Variables ***	**Average levels of constituents**				
		Storage time (Days)				
		0	45	90	135	180
BRS 400	Total phenolics \	9.07±0.12^{aA}	8.59±0.84^{aAB}	8.50±0.25^{aAB}	8.41±0.16^{aB}	8.33±0.13^{aB}
	Total Carotenoids	8.08±0.10^{aA}	7.89±0.13^{aAB}	7.58±0.41^{aB}	7.18±0.12^{aC}	7.05±0.09^{aC}
	Lycopene	19.39±0.09^{aA}	18.98±0.24^{aAB}	18.66±0.21^{aAB}	18.43±0.11^{aAB}	18.10±0.03^{aB}
	β-carotene	6.30±0.27^{bA}	6.06±0.08^{bAB}	5.31±0.83^{bC}	5.54±0.42^{bCB}	5.31±0.13^{bC}
	Antioxidant activity	3.22±0.12^{aA}	3.20±0.06^{aA}	3.05±0.19^{aAB}	2.81±0.31^{aB}	2.82±0.16^{aB}
Cacau	Total phenolics	3.42±0.04^{bA}	3.21±0.03^{bAB}	2.96±0.24^{bAB}	2.69±0.24^{bB}	2.77±0.14^{bAB}
	Total Carotenoids	7.88±0.12^{aA}	7.60±0.21^{aAB}	7.28±0.28^{aBC}	7.21±0.11^{aC}	7.12±0.06^{aC}
	Lycopene	9.22±0.03^{bA}	8.71±0.97^{bAB}	8.77±0.22^{bAB}	8.51±0.24^{bAB}	8.34±0.20^{bB}
	β-carotene	7.20±0.03^{aA}	7.26±0.49^{aA}	6.30±0.21^{aB}	6.26±0.21^{aB}	6.02±0.09^{aB}
	Antioxidant activity	2.32±0.23^{bA}	2.12±0.08^{bAB}	1.88±0.12^{bBC}	1.59±0.18^{bC}	1.62±0.12^{bC}
Jarí	Total phenolics	1.23±0.04^{cA}	1.18±0.07^{cA}	1.33±0.35^{cA}	1.13±0.03^{cA}	1.11±0.02^{cA}
	Total Carotenoids	0.45±0.01^{bA}	0.41±0.01^{bA}	0.29±0.08^{bA}	0.26±0.08^{bA}	0.24±0.04^{bA}
	Lycopene	0.23±0.01^{cA}	0.20±0.01^{cA}	0.15±0.01^{cAB}	0.15±0.04^{cAB}	0.09±0.01^{cB}
	β-carotene	0.43±0.01^{cA}	0.29±0.11^{cA}	0.27±0.08^{cA}	0.25±0.07^{cA}	0.23±0.03^{cA}
	Antioxidant activity	0.50±0.02^{cA}	0.38±0.14^{cA}	0.34±0.11^{cA}	0.31±0.06^{cA}	0.25±0.07^{cA}

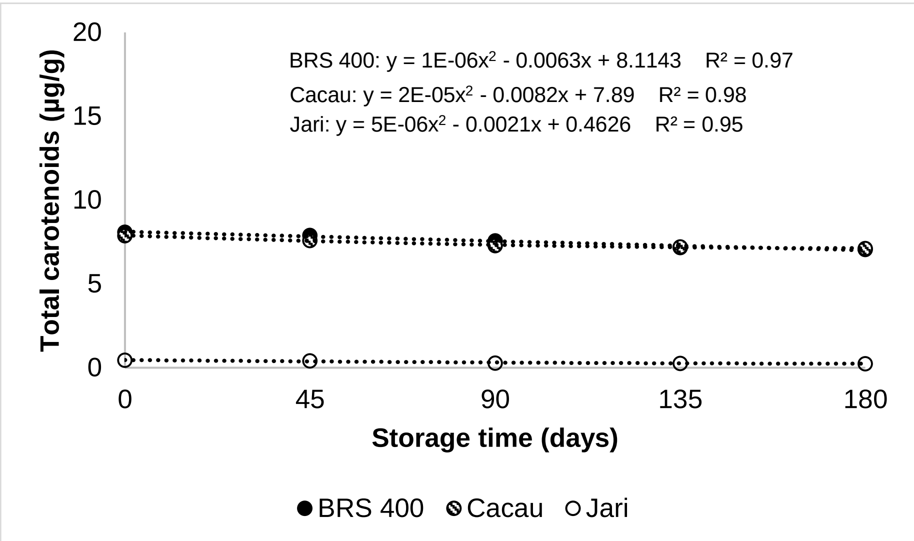
***Legend:** Total phenolics (mg GAE/g extract), total carotenoids (µg/g), lycopene (%), β-carotene (µg/100g), antioxidant activity (SRL%). (1) Values are expressed as mean ± standard deviation. Means followed by the same lowercase letter in the column do not differ between cultivars by the Tukey test (p>0.05). Means followed by the same uppercase letter in the row do not differ between storage times by the Tukey test (p>0.05).

The levels of total phenolic compounds varied significantly among the three cultivars, with BRS 400 showing the highest values at all storage times (P<0.05). The phenolic content in the BRS 400 and Cacau cultivars remained stable for up to 135 days of frozen storage. Although the Jari cultivar exhibited the lowest average phenolic content, no significant losses were observed over the 180-day storage period (Table 1 and Figure 1).



Graph 1. Regression equation and coefficient of determination for the total phenolic levels of cassava cultivars stored for 180 days of freezing.
Source: Research Data

Among antioxidant compounds, phenolics have been widely studied due to their biological activity associated with disease prevention and potential therapeutic effects [12]. Junqueira et al. (2014) [13] reported a reduction in soluble phenols in two cassava cultivars (yellow and white pulp) during 12 days of storage; however, the yellow-pulp cultivar maintained higher phenolic levels than the white-pulp cultivar.
Regarding total carotenoids, the BRS 400 and Cacau cultivars did not differ significantly throughout the storage period and showed higher levels than the Jari cultivar (Table 1). However, significant carotenoid losses were observed in BRS 400 after 90 days, becoming more pronounced at 135 days. In contrast, the Cacau cultivar maintained stable carotenoid levels up to 45 days, while the Jari cultivar preserved its carotenoid content throughout the entire 180-day storage period (Table 1 and Figure 2).
Carotenoids are natural pigments responsible for the yellow, orange, and red coloration of many vegetables. Their molecular structure enables them to react with free radicals, particularly peroxyl radicals and molecular oxygen, which underlies their antioxidant activity [7].



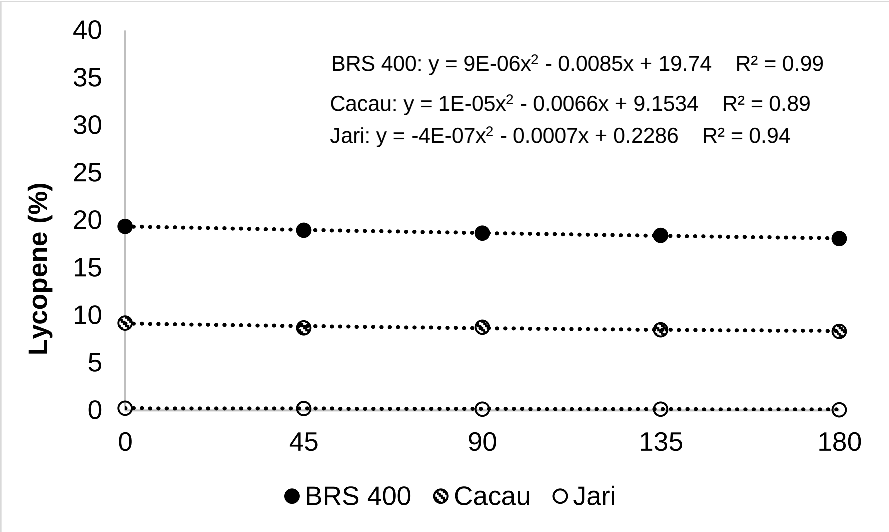
Graph 2. Regression equation and coefficient of determination for the total carotenoid levels of cassava cultivars stored for 180 days of freezing.
Source: Research Data

Junqueira et al. (2014) [13], studying minimally processed cassava cultivars stored at 5 °C for 12 days, observed higher total carotenoid concentrations in the yellow-pulp cultivar compared to the white-pulp cultivar, along with a decrease in carotenoid content over time for both. They also noted that the carotenoid content decreased throughout the refrigerated storage period for both cultivars. According to the authors, this reduction may be attributed to oxidative stress caused by tissue exposure to oxygen during cutting [13]. This finding reinforces that processing and storage conditions influence the stability of these compounds, as also evidenced by the regression graphs.

According to Carvalho et al. (2012) [14], higher carotenoid levels in pink cassava roots, compared to yellow and cream-colored roots, are due to the accumulation of lycopene and β-carotene.

Lycopene content also differed significantly among the cultivars (P<0.05), with BRS 400 showing the highest values throughout the storage period, while Jari exhibited the lowest (Table 1). Lycopene levels remained stable in all cultivars for up to 135 days of frozen storage (Table 1 and Figure 3).

Jacques et al. (2010) [15] attributed lycopene degradation during storage to its molecular structure, which contains eleven conjugated double bonds and two non-conjugated double bonds. This structure confers high singlet oxygen scavenging capacity but also makes lycopene more susceptible to degradation over time.

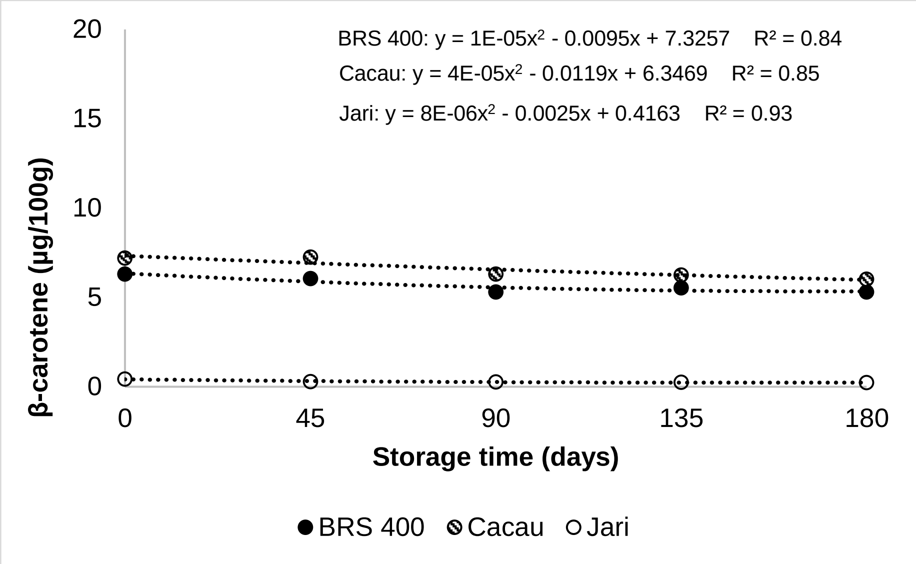


Graph 3. Regression equation and coefficient of determination for the lycopene levels of cassava cultivars stored for 180 days of freezing.
Source: Research Data.

Regarding the β-carotene levels, the Cacau cultivar showed significantly higher values than the other cultivars across all storage times (Table 1) while the Jari cultivar exhibited the lowest average values. In terms of β-carotene losses during storage, this compound remained stable in the BRS 400 and Cacau cultivars only during the first 45 days of freezing, indicating that freezing does not completely prevent degradative processes, which are primarily initiated by tissue disruption during root cutting [2]. In contrast, the β-carotene content in the Jari cultivar did not show significant changes over the 180-day frozen storage period (Table 1 and Figure 4).

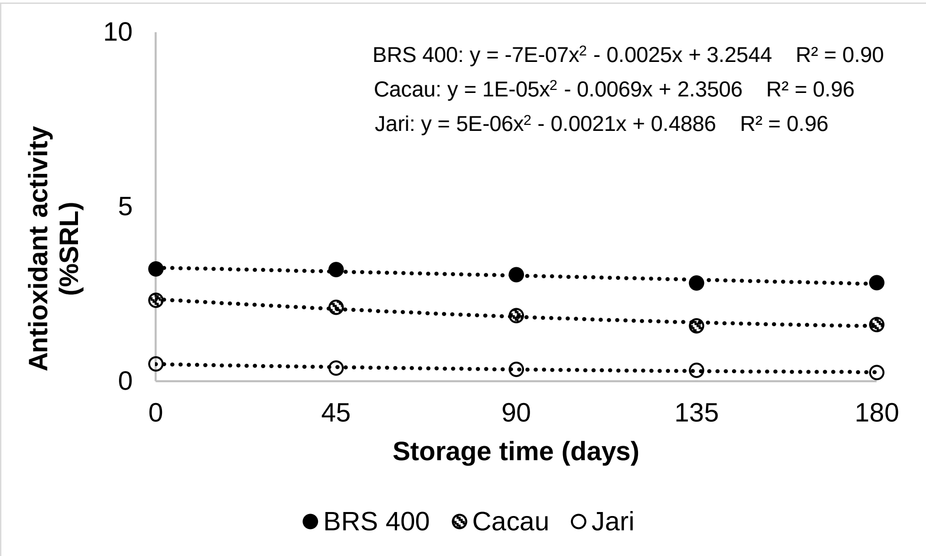
Carotenoids and phenolic compounds are susceptible to oxidative degradation, which can be influenced by factors such as oxygen, light, temperature, and the presence of metal ions [16]. According to Machado, Monteiro, and Tiecher [17], carotenoid degradation occurs through both enzymatic oxidation—initiated immediately after cellular disruption—and non-enzymatic oxidation, which typically involves a lag phase followed by a progressive decline in compound concentration.

Carotenoids play an important role in human nutrition. β-carotene acts as a precursor of vitamin A and is associated with the prevention of conditions such as night blindness and growth disorders, and learning difficulties, while lycopene functions as a potent antioxidant, contributing to the prevention of premature aging and prostate cancer [7].



Graph 4. Regression equation and coefficient of determination for the β-carotene levels of cassava cultivars stored for 180 days of freezing.
Source: Research Data.

Based on the results of free radical scavenging activity, significant differences were observed among all samples during storage, with the highest average values found in the BRS 400 cultivar (Table 1). The Jari cultivar exhibited significantly lower antioxidant activity. With respect to storage time, antioxidant activity remained stable for up to 90 days in BRS 400 and 45 days in Cacau, whereas no significant reduction was observed in Jari throughout the 180-day storage period (Table 1 and Figure 5).



Graph 5. Regression equation and coefficient of determination for the free radical scavenging percentage of cassava cultivars stored for 180 days of freezing.
Source: Research Data.

According to Hoffmann et al. (2017) [18], variations in antioxidant activity and bioactive compound levels are associated with oxidation reactions occurring during processing and storage, reflecting the inherent instability of these compounds.

Similarly, Silva et al. (2014) [7] suggested that changes in bioactive compound levels during frozen storage result from complex interactions influenced by factors such as the food matrix and chemical composition, which affect compound stability in inconsistent ways.

Conclusion

The pink-fleshed cultivar (BRS 400) exhibited the highest levels of phenolic compounds and lycopene, while the yellow-fleshed cultivar (Cacau) showed the highest β-carotene content. In contrast, the white-fleshed cultivar (Jari) presented the lowest levels of bioactive compounds and, consequently, lower antioxidant activity. However, this cultivar showed no significant losses during frozen storage. All three cultivars maintained stable levels of bioactive compounds and antioxidant activity for up to 45 days of storage. Beyond this period, variations in these compounds were observed, depending on the specific characteristics of each cultivar.

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