Biotic compounds, color and antioxidant activity of sugarcane syrup from different varieties cultivated in southern Brazil

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ABSTRACT

The aim of the study was to characterize the bioactive compounds present in sugarcane syrup, analyze the color, to evaluate the antioxidant activity as well as the stability of the bioactive compounds during a 60-day storage under freezing conditions. The experiment was conducted with five sugarcane varieties grown in Canguçu-RS. The content of carotenoids, total phenolic compounds, flavonoids, vitamin C and chlorophylls was determined, and also the antioxidant activity and color were also evaluated. The content of carotenoids (1.82-6.67µg.g), total phenolic compounds (191.06-226.54 mg EAG.L^{-1}), flavonoids (70.73-189.57mg EQ.g^{-1}), vitamin C (0.10-0.35 mg ascorbic acid .100g^{-1}), total chlorophylls (36.13-37.67 g.kg^{-1}), and percentage of antioxidant activity (50.92-68.76%) varied by cultivar. The content of chlorophyll b was higher than the content of chlorophyll a in all cultivars. The color analysis showed that the samples had a strong tendency to green and yellow color. The content of carotenoids increased during 60 days of storage, while the content of phenolic compounds and chlorophylls decreased. The percentage of antioxidant activity decreased after 30 days of storage. It can be concluded that the sugarcane grown in Canguçu-RS had a high content of bioactive compounds, adequate color and high antioxidant activity when compared to the literature.

Keywords: *Saccharum officinarum L.*; phenolic compounds; freezing.

INTRODUCTION

Saccharum officinarum L., popularly known as sugarcane, is a plant cultivated worldwide for over a hundred years and is the raw material for most of the sugar produced in the world. According to the CONAB (2021), 654.5 million tons of sugarcane were grown in Brazil in the 2020/2021 harvest, of which almost 50% were used for sugar production. In addition to sugar, other direct products such as sugarcane juice, molasses, and treacle juice can also be obtained (Ali *et al.*, 2021). According to Sujaritha *et al.* (2017), sugarcane is considered an energy crop because it produces byproducts such as renewable bioelectricity, bioethanol, biosolids, alcohol, chemicals, and fibers that improve environmental sustainability.

The major challenge for sugarcane production in Rio Grande do Sul is due to the climatic characteristics of the region. The state has high humidity, winters with low temperatures and large amounts of rain throughout the year. Therefore, there is a need to develop varieties with improved genetic characteristics, such as RB987935, RB867515, RB925345, RB935744 and RB92579.

Recent studies have reported the presence of phenolic compounds, policosanols, and fatty acids in sugarcane

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(Ali *et al.*, 2021). Antiproliferative, chemopreventive, radioprotective, anticariogenic, and immunoregulatory activities have been observed in studies with sugarcane extracts obtained from leaves, stems, or juice (Barrera *et al.*, 2020). Based on these properties and the content of bioactive compounds, a potential use of sugarcane and its derivatives in the food and nutrition industry can be predicted.

The juice extracted from the sugarcane stalk is popular as a healthy drink in several countries because it is a source of energy and has a sweet taste, so it is well accepted by the public (Kaavya *et al.*, 2019). According to Silva *et al.* (2016), sugarcane juice contains 80% water and 20% total soluble solids (TSS), with a water activity of about 0.99 and a pH > 4.6. The broth appears as a turbid liquid that can form foam, and its color can vary from light gray to dark green, depending on the concentration of ferric salts and tannins present in the broth (Kaavya *et al.*, 2019).

Despite its nutritional value and richness in bioactive compounds, there have been no studies quantifying the bioactive compounds and antioxidant potential of sugarcane juice from varieties grown in southern Brazil. In this context, the main purpose of this study was quantify the bioactive compounds sugarcane juice from different varieties grown in the southern region of Rio Grande do Sul and to evaluate their color, antioxidant activity and the stability of these bioactive compounds during storage under freezing conditions.

MATERIAL AND METHODS

The five sugarcane varieties (RB987935, RB867515, RB925345, RB935744 and RB92579) were grown in the city of Canguçu-RS. The harvest was carried out in March 2022. After harvesting the stems, the juice was extracted in a mill in the city of Pelotas - RS, transported to the laboratory, stored in falcon tubes protected from light, and frozen at -20 ° C at the time of analyses. All analyses were performed in triplicate.

Carotenoid Content

The determination of total carotenoid content was performed according to the method described by Rodriguez-Amaya (2001) with adjustments. A total of 5 g of the sample and 2 g of celite were weighed in, 20 mL of ice-cold acetone was added, and the contents were stirred for 10 minutes. The material was filtered through a cotton cloth and the sample was washed with acetone until it became colorless. The filtrate was transferred to a separatory funnel, where 30 mL of petroleum ether and 30 mL of distilled water were added. The lower phase was discarded, distilled water was added again, and the procedure was repeated 4 times to completely remove the acetone. The upper extract was transferred to a 50 mL volumetric flask andthe volume was made up with petroleum ether. Readings were taken in a spectrophotometer (JENWAY;6705 UV-Vis, SPAIN) at 450 nm, using petroleum ether as a blank. The total carotenoid content was determined according to equation 1 and the results were expressed in µg of β-carotene.g^{-1}of sample.

$$C = \frac{ABS \times vol.\ do\ extrato \times 10^6}{2500 \times 100 \times g\ de\ amostra} \quad (1)$$

Where:
C = total content of carotenoids in the sample;
ABS = absorbance.

Phenolic Compounds

Pure sugarcane juice was used to determine total phenolic compounds. The quantification of the phenolic compounds was carried out according to the methodology described by Singleton & Rossi (1965) by the colorimetric reaction using the reactive solution of Folin Ciocalteau with corresponding reading of the mixture in a spectrophotometer (JENWAY;6705 UV-Vis) at a wavelength of 725 nm. The equation of the straight line expressed by y = 0,0058x-0,1708 and R^2 = 0.9987 was used using gallic acid as standard. The results were expressed in mg gallic acid equivalent per liter of sample (mg EAG.L).

Determination of Flavonoids

The flavonoid content it was determined according to the method proposed by Bueno *et al.* (2016) Sugarcane juice solutions were prepared and homogeneizes with 50% methanol at a ratio of 1:10 (v:v), and then 5 mL of this broth solution was added to 5 mL of $AlCl_3$ (2%) in methanol. This mixture was homogenized and allowed to settle for 30 minutes. Then the absorbance was measured at 415 nm (spectrophotometer Brand: JENWAY; model: 6705 UV-Vis). The curve pattern was determined for the known concentrations of quercetin between 5 and 60 mg.L^{-1} (R^2 0.9997), and the results were expressed in milligrams of quercetin equivalents (mg EQ. g^{-1}).

Vitamin C Content

The vitamin C content of the sugarcane juice was determined by titration with starch, iodine, and sodium thiosulfate solutions prepared previously. Then, the Vitamin C content was calculated (equation 2), and the results were expressed in mg of ascorbic acid.100 g^{-1} sample (Zambiazi, 2010).

$$\text{Vit C}=(Vi\times F1)-(Vt\times F2)$$
$$Y=(Vi\times F1)-(Vt\times F2) \quad (2)$$

Being:

Vi = total spent volume of iodine solution;

F1 = iodine solution correction factor;

Vt = volume of thiosulfate used in the titration;

F2 = thiosulfate solution correction factor;

Where: each mL of 0.01 N iodine solution corresponds to 0.88 mg of ascorbic acid.

Chlorophyll Content

For the analysis of chlorophylls, the method described by Zambiazi (1997) was used, based on weighing 3 g of sample swollen with an isooctane:ethanol (3:1) solution in a 10 mL volumetric flask. Extracts were measured in a JENWAY 6705 UV/VIS spectrophotometer at 630 and 670 nm wavelengths for chlorophyll *a, b* and total. The results were expressed in g. kg^{-1} of sample.

Determination of Antioxidant Activity

The antioxidant activity of sugarcane juice was determined using the DPPH radical according to the method adopted and adapted by Bueno *et al.* (2016), which used a spectrophotometer (brand: JENWAY; model: 6705 UV-Vis) at a wavelength of 515 nm to measure pure sugarcane juice. The results were expressed as the percentage of inhibition of the DPPH ˚ radical (equation 3).

$$\text{Radical scavening activity DPPH (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control Absorbance}} \times 100 \quad (3)$$

Color analysis

The color was determined using a (LOVIBOND PFX880, Germany) colorimeter in the *CI EL*a*b* pattern,* where the L* coordinate expresses the degree of brightness of the measured color (L* = 100 = white; L* = 0 = black), the a* coordinate expresses the degree of variation between red (+60) and green (-60), and the b* coordinate expresses the degree of variation between blue (-60) and yellow (+60).

Stability of bioactive compounds

The content of carotenoids, phenolic compounds, chlorophylls, and antioxidant activity were evaluated after 0, 30, and 60 days of storage of sugarcane juice under freezing conditions (-20 °C). Only the RB925345 variety was used, since it is one of the varieties that stands out in the amounts of bioactive compounds.

RESULTS AND DISCUSSION

Table 1 shows the values of bioactive substances and antioxidant activity of sugarcane juice of the evaluated cultivars RB987935, RB867515, RB925345, RB935744, RB92579.

The total carotenoid content varied significantly between RB987935, RB867515 and RB935744 cultivars, while values were not statistically different between RB925345 and RB92579 varieties ($p > 0.05$).

The cultivar with the highest carotenoid content was RB935744 (6.67 μ g β - carotene. g^{-1}). The results obtained in this study were better than those obtained by Sampaio *et al.* (2022) in the determination of carotenoids in products from sugarcane, where they obtained only 0.73 μg β carotene g^{-1} in molasses. This supports the data that quantities of this bioactive compound are lost during sugarcane processing, justifying the higher value of carotenoids in juice. The carotenoids present in sugarcane juice are found in chloroplasts and are associated with chlorophylls (Schiozer & Cheap, 2013). Their main function in the human body is to act as a precursor of vitamin A (Eggersdorfer & Wyss, 2018).

Variety RB92579 has the highest chlorophyll content (37.67g.kg^{-1}), which is not statistically different from that of other varieties ($p > 0.05$). The variety RB935744 had the lowest content, which is different from the others ($p < 0.05$). The values found are higher than those reported in the literature for juice from sugarcane grown in other regions. According to Begum & Islam (2012), sugarcane juice contained an average only 2.17g.kg^{-1} of chlorophyll, and this content also differed statistically among the analysed varieties. The high chlorophyll content in the juice of the sugarcane varieties in the present study may be related to the maturity of the sugarcane at the time of harvest, as chlorophyll is degraded as the plants mature and senesce. In addition, storing the broth at low temperatures, protected from light and oxygen, may also have contributed to chlorophyll retention (Eskin & Shahidi, 2015).

Table 1: Values of carotenoids, phenolic compounds, flavonoids, vitamin C, chlorophylls and antioxidant activity in the juice of five sugarcane cultivars from Canguçu-RS

Evaluated parameters	**Cultivars**				
	RB987935	**RB867515**	**RB925345**	**RB935744**	**RB92579**
Carotenoids (μg β-carotene. g^{-1})	2.88 ^{c} ± 0.1	3.44 ^{b} ± 0.1	1.82 ^{d} ± 0.04	6.67 ^{a} ± 0.06	1.93 ^{d} ± 0.3
Phenolic compounds (mg EAG. L^{-1})	206.63 ^{b} ± 3.7	204.49 ^{b} ± 4.2	191.06 ^{c} ± 3.7	213.49 ^{b} ± 2.2	226.54 ^{a} ± 5.6
Flavonoids (mg EQ. g^{-1})	119.01 ^{b} ± 0.4	70.73 ^{e} ± 4.6	99.54 ^{c} ± 1.0	189.57 ^{e} ± 0.3	77.19 ^{d} ± 1.3
Vitamin C (mg of L-ascorbic acid.100g $^{-1}$)	0.35 ^{a} ± 0	0.18 ^{c} ± 0	0.14 ^{d} ± 0.01	0.10 ^{e} ± 0	0.23 ^{b} ± 0
Chlorophylls (g.kg $^{-1}$)	37.49 ^{a} ± 0.5	37.35^{b} ± 0.7	36.64^{b} ± 0.3	36.13 ^{b} ± 0.1	37.67 ^{to} ± 0.4
Antioxidant activity by DPPH (% inhibition)	55.45 ^{b} ± 2.8	68.76 ^{a} ± 2.4	53.41 ^{b} ± 0.8	50.92 ^{b} ± 1.5	53.23 ^{b} ± 1.8

Means were compared with ANOVA using Tukey's test at a 5% significance level of 5 %; means marked with the same letter in the row are not statistically different; ± deviation standarddefault.

Chlorophyll *b* content also showed the same behaviour in the studied cultivars (table 2). As for the content of chlorophyll *b* and *a*, there was a significant difference in all the varieties analysed, with the content of chlorophyll *b* being higher in all varieties. The content of chlorophyll *a* and *b* in the present study was much higher than in the study of Begum & Islam (2012), where the highest value for chlorophyll *a* was 1.55 mg.g^{-1} and for chlorophyll *b* was 0,56 mg.g^{-1}. The high content of chlorophyll *b* in relation to the content of chlorophyll *a* can be explained by the conversion of chlorophyll *a* to *b* by the enzyme chlorophyllide-a oxygenase, a reaction that occurs naturally during the ripening of the vegetable (Eskin & Shahidi, 2015).

When analyzing the color in the juice of the sugarcane cultivars in the present study (Table 3), cultivar RB987935 obtained the highest value for the parameter L* ($p < 0.05$), while cultivar RB92579 obtained the lowest value for the parameter luminosity. Regarding the parameter a*, the cultivars were statistically equal. The a* value expresses the degree of variation between red (+60) and green (-60). Therefore, the sugarcane juice of the cultivars in the present study showed a high tendency to green color. As for the parameter b*, the RB925345 variety had the highest value. Based on the data for this parameter, the sugarcane juice of the cultivars in the present study also showed a high tendency to yellow.

In the study of Brochier *et al.* (2018), sugarcane juice had a higher value for the luminosity parameter, i.e., a lighter hue (L* 31.3 ± 0.2); but also, with a tendency toward green (a* 1.08 ± 0.07) and yellow (b* 1.71 ± 0.07). The development and change of colors in sugarcane juice are due to the presence of natural pigments, enzymatic activities, and Maillard reactions, among other factors. Some authors have linked color to the presence of bioactive compounds and antioxidant activity, such as phenolic compounds (Brochier *et al.*, 2018; Duarte-Almeida *et al.*, 2011; Sant'anna *et al.*, 2013).

In terms of the number of phenolic compounds, the variety with the highest contents was RB92579 (226 .54 mg EAG. L^{-1}), while the lowest values were observed in variety RB925345 (191.06 mg EAG.L^{-1}). There were no significant differences in the content of phenols in the other three cultivars (RB987935, RB867515, RB935744). Their results are in agreement with other studies, such as Sreedevi *et al.* (2018), who found values between 30.2 and 35.2 mg EAG.100 mL in sugarcane juice taken under pressure and frozen.

Table 2: Values of chlorophyll a and chlorophyll b in juice of five sugarcane cultivars from Canguçu-RS

Cultivars	**Mean values (g.kg^{-1}) ± Desvio padrão**	
	Chlorophyll *a*	**Chlorophyll *b***
RB987935	13,38^{b} ± 0,1	23,18^{a} ± 0,1
RB867515	13,37^{b} ± 0,1	23,16^{a} ± 0,2
RB925345	12,65^{b} ± 0,2	21,92^{a} ± 0,4
RB935744	12,52^{b} ± 0,2	21,68^{a} ± 0,4
RB92579	13,34^{b} ± 0,4	23,11^{a} ± 0,7

The values were compared using the T-test for dependent groups at the significance level of 5% ($p < 0.05$). Averages followed by the same lowercase letter in the row do not differ statistically.

Table 3: Color parameters in juice of five sugarcane cultivars from Canguçu-RS

Color Parameters	RB987935	RB867515	RB925345	RB935744	RB92579
L*	17.32 ^{a}± 0.32	17.02 ^{ab}± 0.06	17.21 ^{a}± 0.10	16.66^{b}± 0.33	16.41 ^{b}± 0.44
A*	-0.63 ^{a}± 0.05	-0.67 ^{a}± 0.04	-0.75 ^{a}± 0.12	-0.68 ^{a}± 0.08	-0.64 ^{a}± 0.02
B*	4.14 ^{c}± 0.11	4.31 ^{bc}± 0.06	4.6 ^{a}± 0.09	4.27 ^{c}± 0.06	4.56 ^{ab}± 0.17

Means values were compared with ANOVA using Tukey's test at a significance level of $p < 0.05$. Means values marked with the same letter in the row are not statistically different; ± deviation standard.

The flavonoid content varied significantly among the five cultivars analyzed. The highest amount of flavonoids was found in cultivar RB935744 (189.57 mg EQ. g^{-1}), while the cultivar with the lowest bioactive flavonoid content was RB867515 (70.73 mg EQ. g^{-1}). The result of flavonoids was higher than that reported in the literature for sugarcane in other regions. Ali *et al.* (2021) determined the content of flavonoids in sugarcane juice, obtained only 2.59mg.g. The flavonoid content shows that it correlates with the compound content of phenols and color (Sampaio *et al.*, 2022). The potential nutraceutical sugarcane juice is determined on the basis of its flavonoid content represented by apigenin, luteolin, diosmetin, vitexin, tricin and asterin (Maia, 2022). This class of bioactive has been associated with various health effects over time, such as anti-inflammatory, antioxidant, antimutagenic and anticancer activity (Panche *et al.*, 2016).

According to the literature, the phenolic compounds present in sugarcane stalks are mainly caffeic, chlorogenic and coumaric acid, although this profile may vary depending on the variety (Duarte-Almeida *et al.*, 2011). This group of phytochemicals has been studied for their antitumor, anti-inflammatory, antioxidant, and antihistamine activities (Singh *et al.*, 2018).

Vitamin C content differed significantly among all cultivars analyzed in the present study. The highest content of ascorbic acid was RB987935 (0.35 mg of L -ascorbic acid.100 g^{-1}), while the variety with the lowest value was RB935744 (0.10 mg of L -ascorbic acid.100 g^{-1}). For the analysis of sugarcane juice stored for 21 days under freezing conditions, Zia *et al.* (2019) obtained a value of 0.3 mg of acid. L-ascorbic.100 g^{-1} in the samples evaluated. This shows that the acidity of ascorbic acid is degraded with increasing storage time. The low amount of ascorbic acid is due to storage conditions since the presence of oxygen is essential to the bioactive degradation of this compound (Aguilar *et al.*, 2017).

The percentage of antioxidant activity was highest in cultivar RB867515 (68.76% inhibition), and the cultivar with the lowest antioxidant activity was RB935744 (50.92% inhibition), which statistically behaved the same in too many cultivars. The results are consistent with other studies, such as that of Zia *et al.* (2019), which obtained a value of 61.2% inhibition after 21 days of freezing when determining the activity antioxidant in sugarcane juice. It is significant activity antioxidant presented suggests the use of sugarcane derivatives by the industry nutraceuticals in the treatment of diseases related to free radicals in the body human.

Table 4 shows the behavior of bioactive compounds in sugarcane juice of RB925345 variety at 0, 30, and 60 days of storage under freezing conditions. As shown in Table 4, the carotenoid content increased by 19% after 60 days of storage. The high content was expected, as the sample showed a strong tendency to yellow color. Silva *et al.* (2015), also observed an increase in carotenoid content after 30 days of storage of araticum fruit pulp under freezing conditions, showing, that changes in carotenoid content during storage under freezing conditions are due to complex processes influenced by several factors, such as food matrix, chemical composition, and others.

The phenolic compounds content decreased by 27% during the 60 days of storage. The research of Machado *et al.* (2019), who stored *Physalis sp.* pulp under freezing conditions, also observed losses over the 120-day storage period, in which phenolic compounds phenolics showed a decrease of 22.26% and 31.31% of the original content in the unpasteurized and pasteurized squashes, respectively.

The chlorophyll content also showed a decrease of 7% during the 60 days of storage. The loss of partial chlorophyll was already expected for increased carotenoid content. This occurs due to the ripening process of vegetables, where chloroplasts transform into chromoplasts and these lose chlorophyll and initiate carotenoid synthesis (Eskin & Shahidi, 2015). In addition, due to the high instability of chlorophylls, even at low temperatures, the process of chlorophyll degradation can be in their derivatives, because of the components on the food matrix and storage conditions.

Table 4: Bioactive substances content and antioxidant activity in the juice of the sugarcane cultivar RB925345, from Canguçu-RS, evaluated over a period of 60 days under freezing conditions (-20 ° C)

Parameters evaluated	Period (days)		
	0	30	60
Carotenoids (µg β- carotene . g $^{-1}$)	1.82 ^{c} ± 0.04	2.08 ^{b} ± 0.04	2.17 ^{a} ± 0.02
compounds phenolics (mg EAG. L $^{-1}$)	185.31 ^{a} ± 22.71	150.98 ^{b} ± 4.03	135.33 ^{c} ± 0.52
Chlorophylls (g.kg $^{-1}$)	36.64 ^{a} ± 0.28	35.32 ^{b} ± 0.41	34.28 ^{c} ± 0.16
Activity DPPH antioxidant (% inhibition)	53.41 ^{b} ± 0.77	53.02 ^{b} ± 0.73	55.99 ^{a} ± 0.13

Means were compared with ANOVA using Tukey's test at a 5% significance level; means marked with the same letter in the row are not statistically different; ± deviation standard.

The antioxidant activity was found to be stable up to a storage period of 30 days. However, after 60 days, an increase of 5% was shown. Carvalho *et al.* (2017) to evaluate the juice stability of tropical mixture stored under freezing, also observed oscillations in antioxidant activity, concluding that freezing storage would be a preservation method effective in maintaining the functions of chemical and activity samples antioxidant analyzed.

CONCLUSIONS

It is concluded that the chemical composition and antioxidant activity of sugarcane syrup are affected by the varieties, even when grown in the same microregion (Canguçú - RS), showing that the properties are specific to the genetics of each variety. However, all cultivars grown and analyzed showed increased bioactive composition and showed a high percentage of antioxidant activity compared to cultivars from other regions. In addition, the bioactive compounds were found to be stable when frozen for 60 days, as the variations in the content of the bioactive compounds studied were very low. As a result, sugarcane grown in the region show great potential for use in the food and pharmaceutical industries.

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