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Isotonic sports drink prepared from pineapple juice: Stability during its accelerated storage

Bebida deportiva isotónica a base de jugo de piña: Estabilidad durante su almacenamiento acelerado

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ABSTRACT

It was evaluated the behavior, during accelerated storage, of the quality indicators of an isotonic sport drink made from the mixture of pineapple juice, distilled water, sucrose and NaCl. The drink was packed in amber glass bottles was pasteurized at 80 °C for 5 min in a water bath. The drink was stored at 45, 50 and 55 °C with the aim of achieving accelerated deterioration. The drink presented a total sugar content of 7.6%, osmolality of 328 mOsm/kg and sensory acceptance corresponding to the category -I like it slightly-. At the end of storage at each temperature, there was a decrease in brightness and an increase in the values of a* and b*, related to the increase ($p \leq 0.05$) of the browning index (IP) and the moment of sensory rejection, due to a notable loss of pineapple color. The behaviors of the IP at each temperature were adjusted to polynomial models of order five with high coefficients of determination (> 0.98). This study provides useful information for the optimization of beverage production, quality control and spoilage prediction, which could contribute to the development of new products and satisfy consumer needs.

Keywords: pineapple; food preservation and processing; food quality; kinetics; sensory properties.

RESUMEN

Se evaluó el comportamiento, durante almacenamiento acelerado, de los indicadores de calidad de una bebida deportiva isotónica elaborada a partir de la mezcla de jugo de piña, agua destilada, sacarosa y NaCl. La bebida se envasó en botellas de vidrio color ámbar y se pasteurizó a 80 ºC durante 5 min en baño María. La bebida se almacenó a 45, 50 y 55 ºC para conseguir su deterioro acelerado. La bebida presentó un contenido de azúcar total de 7,6%, osmolalidad de 328 mOsm/kg y aceptación sensorial correspondiente a la categoría -Me gusta un poco-. Al final del almacenamiento a cada temperatura, hubo una disminución del brillo y un aumento en los valores de a* y b*, relacionado con el aumento ($p \leq 0,05$) del índice de pardeamiento (IP) y al momento de rechazo sensorial, debido a una notable pérdida del color a piña. Los comportamientos del IP a cada temperatura se ajustaron a modelos polinómicos de orden cinco con coeficientes de determinación altos (> 0,98). Este estudio proporciona información útil para la optimización de la producción de la bebida, control de calidad y predicción del deterioro, que pudieran contribuir al desarrollo de nuevos productos y satisfacer las necesidades de los consumidores.

Palabras clave: piña; conservación y procesamiento de alimentos; calidad de los alimentos; cinética; propiedades sensoriales.

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1. Introduction

The water balance of the organism at rest is maintained thanks to an adequate balance between the inflow and outflow of fluids. However, when doing physical exercise, you should replace lost fluids before symptoms of dehydration occur, as this can affect the performance and increase the risk of injury (Ruiz & Garcia, 2022; Muñoz-Urtubia et al., 2023). Research agrees that isotonic drinks, consumed before, during, and after prolonged and intense exercise or other strenuous physical activity, are more effective than water in preventing dehydration, helping to maintain performance during exercise, delaying onset of fatigue and speed recovery (Shalesh et al., 2014).

Commercial sports drinks often contain synthetic ingredients to improve palatability and prolong shelf life, but have been questioned for possible harm to consumers' health (Orrù et al., 2018). Based on the demand for more natural drinks, there is an interest in the food industry, to produce isotonic drinks from fruit juices, due to they not only add natural flavor and color, but also provide vitamins, minerals and antioxidant substances that enrich, from a sensory and nutritional point of view, the formulation of this type of product.

In this sense, pineapple (*Ananas comosus* L. Merrill), a tropical fruit whose juice contains a large amount of water and some minerals such as potassium, magnesium, and phosphorus; in addition to being an excellent source of vitamin C, can be used in the formulation of an isotonic drink (Ruiz et al., 2018).

During the processing and storage of fruit-derived products, non-enzymatic browning reactions occur that deteriorate their sensory and nutritional characteristics. Even though some papers have stated the use of fruits for preparing isotonic drinks (Bendaali et al., 2022; Bendaali et al., 2023), the available data about their storage behavior, are still limited. Therefore, this research assessed the evaluation of the behavior of the physical, chemical and sensory indicators of a pineapple juice-based isotonic sports drink during accelerated storage.

2. Material and methods

2.1 Obtaining the clarified pineapple juice

Healthy and ripe pineapples (*A. comosus* L. Merrill) purchased from the same market were used. The crown was removed, and they were washed and disinfected with drinking water and sodium hypochlorite solution (80 mg/L), respectively; then, they were dried at ambient temperature, and relative humidity.

After peeling and cored, they were cut into small pieces, which were ground in a Grindomix grinder (Mod. GM 200, Germany). The pulp was pressed to obtain a juice with a certain degree of clarification (13.58% of total solids; 13.41% of total sugars; 1.57 mg/100 g of sodium; 131.30 mg/100 g of potassium and an osmolality of 628 mOsm/kg).

2.2 Drink preparation

For the preparation of the drink, pineapple juice, sucrose (Uni-Chem International, India) were used as raw materials; sodium chloride (Uni-Chem International, India) and distilled water with an osmolality of 68 mOsm/kg.

For its elaboration (Figure 1), the necessary volumes of each one of the raw materials were weighed in a technical balance or measured. Then, the pineapple juice (34.0%), distilled water (62.8%), sucrose (3.1%) and NaCl (0.1%; 20 mmol/L) were mixed by mechanical stirring until its total dilution. The drink was packed in 350 mL amber glass bottles, previously washed and sterilized, with crown-type plates. Finally, the product was pasteurized at 80 °C for 5 min in a water bath (Scientz SC-15, China).

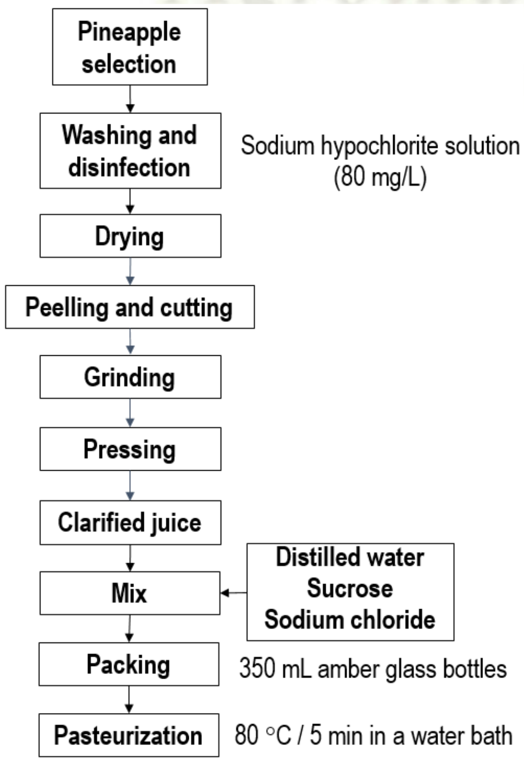

Figure 1. Operations for the preparation of the isotonic sports drink.

2.3 Characterization of the isotonic sports drink

For the evaluation of the isotonic sports drink, proteins (AOAC, 2019) and sucrose (AOAC, 2019) were determined. Titratable acidity was expressed as percentage w/w of citric acid.

The soluble solids content was measured by refractometry. The results were expressed as ºBrix. Temperature and acidity corrections were applied to the obtained values. In addition, the soluble solids / acidity ratio was calculated as an indicator of the flavor of the product.

The total sugar content was found by the phenol-sulfuric acid method (Sewwandi et al., 2020). The method is based on the fact that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. The subsequent reaction between these derivatives and phenol develops a detectable color.

The procedure followed was as follows: a 2 mL aliquot of previously diluted juice (10-4 dilution) was mixed with 1 mL of 5% aqueous phenol solution in a test tube. Then, 5 mL of concentrated sulfuric acid was quickly added to the mixture. After the tubes were allowed to stand for 10 minutes, they were shaken for 30 s and placed for 20 min in a room temperature water bath. Absorbance at 490 nm was determined on the resulting colored solution in a spectrophotometer (Ray-Leigh UV-1601, China).

For the calibration curve, sucrose was used as a standard at concentrations between 0.01 and 0.05 g/L. A blank test prepared with distilled water was also performed under the same conditions as the sample, to calibrate the equipment and eliminate interferences due to the solvents and reagents used in the art. The total sugar content was expressed in % w/v.

The mineral content was determined by atomic absorption spectroscopy (Alves et al., 2019). The results were expressed in mg/100 g. Vitamin C was determined by the volumetric method of 2,6-dichlorophenolindophenol (Muhammad, 2016) and the results were expressed in mg/100 g.

Osmolality was determined with a vapor pressure osmometer (Wescor Vapro 5520, USA) calibrated with solutions of 100, 290 and 1000 mmol/kg. For reading 10 μL of sample previously centrifuged (Eppendorf 5804 R, Germany) was used for 5 min at 10000 min^{-1}.

The general acceptance of the drinks was determined by applying an affectionate sensory level test with the participation of 60 potential consumers. A 9-point verbal hedonic scale was used from "I like extremely" to "I extremely dislike". The encoded samples were served at a temperature of 15 °C. For processing, the verbal scale was converted to a numerical scale where 1 corresponded to the category "I extremely dislike" and 9 to the category "I extremely like it".

2.4 Evaluation of the physical and chemical indicators of isotonic sports drink during storage

The bottled drinks were stored in an AISET YLD-6000 incubator (China) at 45, 50 and 55 °C with the aim of achieving accelerated deterioration. During storage, pH and colorimetric parameters L*, a*, b* and browning index (IP) were determined.

The L*, a* and b* color coordinates were determined using the spectrophotometric method according to the recommendations of the International Commission on Illumination (Ruiz et al., 2017). For this, the juice was centrifuged (Eppendorf 5804 R, Germany) at 5000 min^{-1} for 20 min. The supernatant was transferred to glass cuvettes and the absorbance in the visible electromagnetic radiation spectrum (400 to 700 nm) was determined at 10 nm intervals with a Ray-Leigh UV-1601 spectrophotometer (China). A blank was prepared with distilled water, the absorbance values of which were subtracted from those of the sample.

The absorbance data was transformed into L*, a* and b* coordinates of the CIE L* a* b* three-dimensional color model, using as reference the CIE D_{65} Standard Illuminant and Standard Observer (10° visual angle). The three parameters of this model represent the luminosity or brightness of the color (L*, L= 0 black, L= 100 white), its position between red and green (a*, a < 0 green, a > 0 red) and its position between yellow and blue (b*, b < 0 blue, b > 0 yellow).

2.5 Browning index

The conversion of the L*, a* and b* coordinates to the XYZ tristimulus values allowed calculating the browning index defined as (Shrestha et al., 2020):

$$IP = \frac{(x - 0.31)}{0.172} * 100 \quad \text{(Eq. 1)}$$

Where x is the chromaticity coordinate calculated from the XYZ values according to x = X/(X + Y + Z).

2.6 Quantitative descriptive analysis

The sensory characteristics of the drink were evaluated during its storage at 45, 50 and 55 °C by means of a quantitative descriptive analysis.

The descriptor generation process was carried out with seven evaluators trained in this type of product, using the controlled association method. The deletion of the terms was carried out in open discussion with the judges. The sensory descriptors of the product were evaluated on a structured 10-cm scale bounded at both ends with increasing intensity of the descriptor from left to right, as indicated by the method of quantitative descriptive analysis.

2.7 Analysis of the results

The results were subjected to an analysis of variance using the STATISTICA program (version 7, 2004, StatSoft. Inc., Tulsa, USA). When a significant difference ($p \leq 0.05$) was detected, Duncan's multiple range test was applied to compare the differences between the samples.

3. Results and discussion

3.1 Characterization of the isotonic sports drink

Table 1 shows the results of the chemical and composition indicators of the isotonic sports drink from pineapple juice. In general, the isotonic sports drink presented a composition in accordance with that of other similar drinks. The isotonic sports drink presented an osmolality of 328 mOsm/kg and a sensory acceptance that corresponded to the category - I like it slightly.

Table 1
Characterization of the isotonic sports drink from pineapple juice

Parameter	Mean (standard deviation)
Proteins (%)	0.34 (0.02)
Titratable acidity (%)	0.18 (0.01)
Soluble solids (°Brix)	6.19 (0.2)
Soluble solids/acidity	34.4 (0.3)
Sucrose (%)	3.13 (0.07)
Sodium (mg/100 g)	39.87 (0.01)
Potassium (mg/100 g)	44.64 (0.05)
Calcium (mg/100 g)	15.62 (0.04)
Vitamin C (mg/100 mL)	9.83 (0.89)
Total sugars (%)	7.6 (0.5)
Osmolality (mOsm/kg)	328 (6)
Sensory acceptance	6.2 (0.6)

Other sports drink has been designed from pomegranate (*Punica granatum*) and garcinia (*Garcinia indica*) (Vaibhavi and Bhakti, 2014), obtaining an isotonic product with 6.33% carbohydrates; 105.6 mg/100 mL of vitamin C; 33.99 mg/100 mL of potassium; 65.75 mg/100 mL of sodium; 4,085 mg/100 mL of phosphates; in addition to chloride, calcium, magnesium, and iron. It should be noted that differences could be due, among other factors, to the composition of the fruits used and processing operations to obtain the drinks.

Various natural products have also been studied for their rehydrating properties, which sometimes include minor modifications in their composition. The effectiveness of water, a commercial sports drink, coconut water, and sodium-enriched coconut water in rehydrating and restoring plasma volume after exercise-induced dehydration has been compared. As result, sodium enriched coconut water showed a rehydrating effect similar to that of sports drink (Ismail et al., 2007).

Consumers who participated in sensory acceptance tests were not regular consumers of this type of product. This contributed to the fact that the drink was sometimes rejected as it was evaluated under the quality standards of fruit juices. It is possible to improve the acceptance by adding sweeteners that have a minimal contribution to the osmolality of the drink.

3.2 Behavior of physical and chemical indicators of isotonic sports drink during storage pH value

The pH plays an important role in the taste of fruit drinks, also acting as a conservation factor. This parameter is considered one of the main indicators of the stability of bioactive compounds, and the quality of these products (Hashem et al., 2014).

Table 2 presents the variation in the pH of the isotonic sports drink during accelerated storage. During storage there were slight variations in pH, without showing a tendency to increase or decrease and which are of little importance from a practical point of view. This result coincided with what was reported (Muhammad, 2016) for pineapple drinks and juices.

Table 2
Behavior of pH of isotonic sports drink during accelerated storage

Storage time (d)	Temperature (ºC)		
	45	50	55
0	3.58 (0.04)^{ab}	3.58 (0.04)^{ab}	3.58 (0.04)^{ab}
5	3.65 (0.00)^{cd}	3.62 (0.05)^{bc}	3.64 (0.00)^{cd}
7	3.69 (0.02)^{de}	3.66 (0.006)^{cde}	3.66 (0.00)^{cde}
8	3.65 (0.02)^{cd}	3.64 (0.03)^{cd}	3.56 (0.02)^{a}
9	3.7 (0.1)^{e}	3.65 (0.03)^{cd}	3.67 (0.02)^{cde}
10	3.58 (0.00)^{ab}	3.58 (0.006)^{ab}	-
11	3.61 (0.02)^{abc}	-	-

Mean (standard deviation); n = 3.
Different letters indicate significant differences at $p \leq 0.05$.
-: The product was rejected at that time.

It has been suggested that small variations in pH could be due to the occurrence of biochemical reactions during storage periods (Muhammad, 2016). However, the pH remained between 3.56 and 3.7; an important factor considering that the growth of *Clostridium botulinum*, and other microorganisms of sanitary interest is inhibited at pH values <4.5, while below 3.7, only molds and yeasts can develop (Kim et al., 2018).

3.3 Color coordinates L*, a* and b*

Figure 2 shows the evolution of the color parameter L* during the storage of the drink at 45, 50 and 55 ºC. During storage, the luminosity of the drink varied, in general way, in all cases, between values of 90 and 95. This variation in luminosity over time showed a similar behavior for each storage temperature. Initially, a slight increase was observed, to then remain stable and decrease at the end of storage. The end of the sampling coincided with the days when the drink was rejected by the trained judges. The changes in the color of the isotonic drink during storage were a direct manifestation of the evolution of non-enzymatic browning reactions and changes in the pigment content, among other factors.
Similar to the behavior observed in the present work, a passion fruit juice, stored at 25 °C for 120 days, presented an increase in L* at the beginning of storage and a subsequent decrease in the value of this parameter (Sandi et al., 2004). In this sense, a sudden decreases in luminosity can occur from one sample to another. On the other hand, a decrease in luminosity was also reported during the storage of unpasteurized pineapple juice (Laorko et al., 2013).
In pineapple, the color is mainly due to the presence of the xanthophylls lutein and zeaxanthin, which are carotenoid compounds (González-Peña et al., 2023). An increase in luminosity in the first days of storage can be caused by the modification of the carotenoid structure, resulting in a lighter color. Over time, other compounds, resulting from fundamentally non-enzymatic browning reactions, or the precipitation of pigments, would contribute to the reduction of luminosity, giving the product a darker appearance (Silva et al., 2006).
Figure 2 also shows the behavior of the color coordinates a* and b* of the isotonic sports drink at the three storage temperatures. The drink showed significant changes ($p \leq 0.05$) of the color parameter a*, with a similar behavior for all temperatures. Initially, there was a slight decrease, on the intermediate days it remained relatively stable and on the last day, it increased, coinciding with the sensory rejection of the drink.

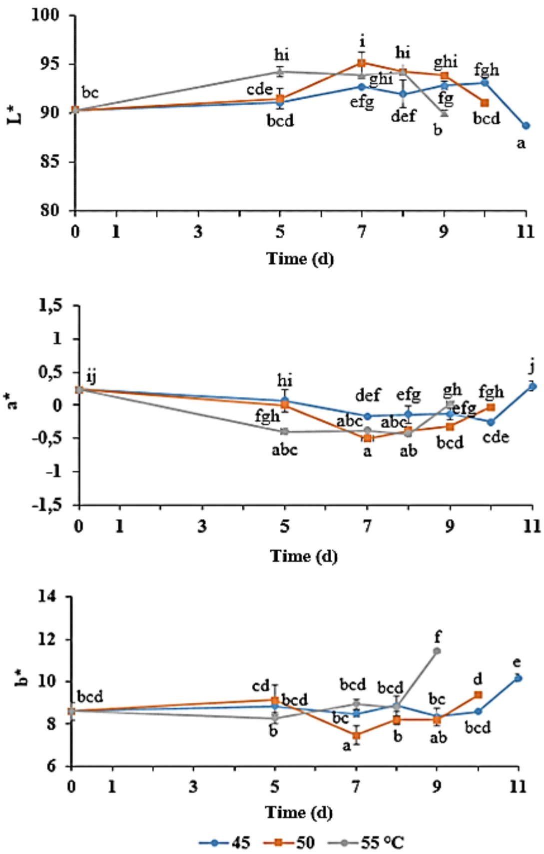

Figure 2. Behavior of the color coordinates of the isotonic sports drink during accelerated storage. The error bars represent the standard deviation (n = 3). Different letters indicate significant differences at $p \leq 0.05$.

The decrease of a* until reaching negative values implied a shift from a reddish hue to a greenish one, that is, it was a slight discoloration. A particular characteristic of the carotene structure is the presence of a conjugated double bond system that acts as a chromophore, giving these pigments yellow, orange and red (Meléndez-Martínez et al., 2019). These double bonds can undergo cis-trans isomerization, which occurs during the incidence of light and temperature (Hock-Eng et al., 2011). This isomerization could lead to a loss of color (Khoo et al., 2011).
The b* coordinate, which represents the yellow color of the product, was maintained with values between 8 to 9 in general, without the variations being important from a practical point of view regarding color perception ($p > 0.05$) throughout storage, to then show an increase in the last sampling for each of the temperatures.

In studies of storage of fruit drinks, there is great diversity in the evolution of a* and b* coordinates, depending on the type of fruit, storage conditions, among other factors. Thus, for example, Laorko et al. (2013) obtained negative slopes of a*, while during storage at 7 ºC of a pineapple juice with small pieces of fruit, no variations were found in the value of this parameter (Tobolková et al., 2013). Furthermore, when storing mango nectars with high-intensity sweeteners at 25 °C for 120 days, a significant increase in a* and b* values was observed (Cadena et al., 2013).
The changes in the a* and b* coordinates should not be analyzed separately, as together they define the chromaticity of the tone of the product (Ruiz et al., 2017). The results indicated that, during storage, the tone practically did not change, but on the last day a more yellow and reddish tone was obtained, which corresponds to a brown color. Taken together, the changes in L*, a* and b* at the end of storage could be induced by non-enzymatic browning reactions, such as the Maillard reaction and degradation of ascorbic acid, with the formation of compounds that impart a brown color to the product.

3.4 Browning index

Many of the non-enzymatic browning reactions are oxidative processes involving substances found in pineapple juice-based isotonic drinks, such as sugars and ascorbic acid. The browning index (IP) is an indicator of the darkening of the product that occurs as result of the formation of brown compounds during these reactions, and includes in its calculation the three color coordinates L*, a* and b*.
Different colorimetric indicators have been used to study non-enzymatic browning in fruit drinks, such as the measurement of absorbance at 420 nm (Bork et al., 2022); brightness and color difference (Moon et al., 2020) and IP (Shrestha et al., 2020). This index is defined as the purity of the brown color.
Figure 3 shows the behavior of the IP during the accelerated storage of the drink. The behavior of the IP during storage summarizes, in some way, the behavior of the color coordinates L*, a* and b*. At the beginning of storage, the IP showed a slight decrease, followed by a period of stability and an increase on the last day of sampling for each of the temperatures; this behavior was more accelerated at a higher storage temperature.
At the end of storage, the color of the drink changed abruptly, turning brownish. This was a key factor in the sensory evaluation of the product, since the deterioration of the color was what determined its rejection by the judges.

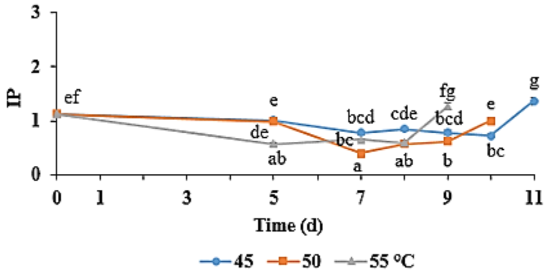

Figure 3. Behavior of the browning index (IP) of the isotonic sports drink during accelerated storage. The error bars represent the standard deviation (n = 3). Different letters indicate significant differences at p ≤ 0.05.

The equations for the evolution of the IP allow determining its relationship with the accelerated storage time (Table 3). The behavior of the IP during storage was adjusted, for all temperatures, to a polynomial model of order five with determination coefficients of 0.990, 0.992 and 1.00 for 45, 50 and 55 °C, respectively. This indicated the good fit of the theoretically obtained equations to the experimental data, that is, the models efficiently describe the relationship between the two variables under study.

Table 3
Polynomial models for the behavior of the browning index during accelerated storage of the isotonic sports drink

Temperature (ºC)	Model	R^2
45	$y= 0.0008x^5-0.0256x^4+0.2889x^3-1.47x^2+2.5992x+1.1225$	0.990
50	$y= 0.0002x^5-0.0082x^4+0.1228x^3-0.7993x^2+1.7874x+1.1228$	0.992
55	$y= 0.0036x^5-0.0935x^4+0.8842x^3-3.6305x^2+5.3448x+1.1231$	1.000

3.5 Quantitative descriptive analysis of isotonic sports drink during storage

Figure 4 shows the quantitative descriptive profiles of the isotonic sports drink during storage. Ten typical descriptors of fruit drinks were generated. The aroma plays an indisputable role in the acceptance and choice of food; whether an undesirable aroma is perceived or not, the rejection of the product corresponds to the expectations of the consumer.

Initially, the pineapple odor of the drink was intense (7.8 out of 10) and no cooked odor was perceived (0 out of 10). During storage, the pineapple odor decreased, and the cooked odor increased, a situation that began to be more noticeable from the seventh day at 50 and 55 °C and from the ninth day at 45 ºC. During storage, no strange odors were perceived.

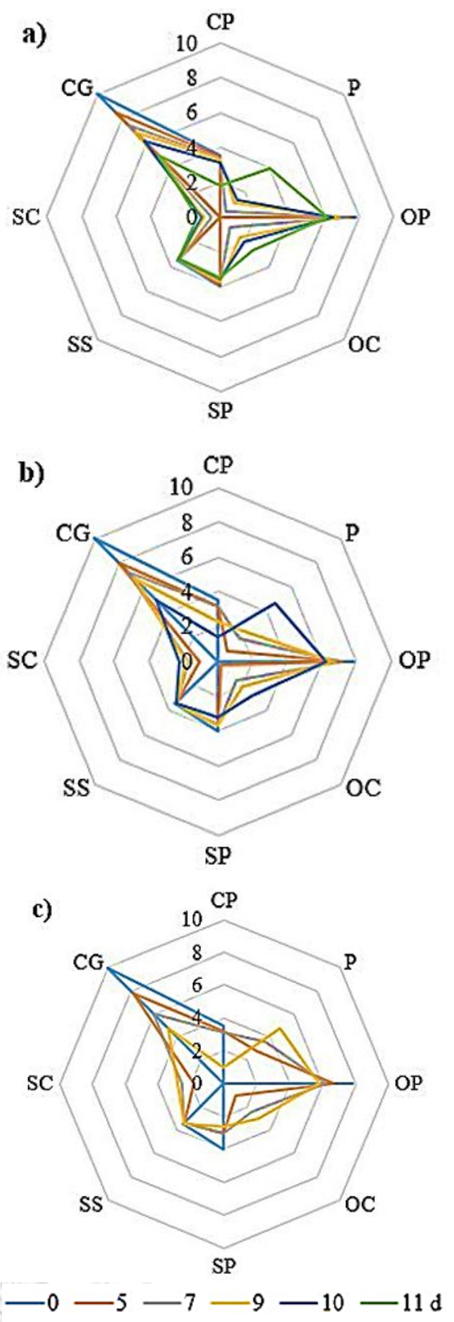

Figure 4. Quantitative descriptive profiles for the isotonic sports drink during accelerated storage at a) 45; b) 50 and c) 55 ºC. CP: pineapple color; P: browning; OP: pineapple odor; OC: cooked odor; SP: pineapple taste; SS: salty taste; SC: cooked taste; CG: global quality.

In the case of flavor descriptors, the behaviors of pineapple flavor and cooked flavor were related. Initially, the drink had a medium pineapple flavor (4 out of 10), a slight salty taste (3.5 out of 10) and no cooked flavor (0 out of 10). During storage, the pineapple flavor decreased while the cooked flavor increased, but in both cases, there were small changes. No foreign flavors were detected, and the salty taste remained unchanged.

The aroma and flavor changes could have been the result of non-enzymatic browning reactions. The flavor compounds are molecules that can be volatile or non-volatile, which when entering the mouth volatilize and generate aromas; they are derived primarily from the hydrolysis of proteins, carbohydrates, lipids, ribonucleotides, and pigments. The aroma compounds are low molecular weight volatile compounds, generated by lipid oxidation, Maillard reactions, caramelization, degradation, and by the interaction of the products of these reactions (Liu et al., 2022).

The color profile was analyzed based on two descriptors, pineapple color and browning. During storage, the color to pineapple faded as lighted browning appeared. This trend was most notable on days nine, ten, and eleven for 55, 50 and 45 °C, respectively; which implied the rejection of the drink by the judges.

It is notable that the behavior of the IP during storage was not related to the evolution of the browning descriptor of the quantitative descriptive analysis. This could occur because, in the determination of L*, a* and b*, the drink was previously filtered, which eliminated the suspended particles, while this operation was not performed for sensory evaluation. However, the moment of sensory rejection, given by a loss of yellow color and intense browning, coincided with the moment in which the IP increased significantly ($p \leq 0.05$) at each of the temperatures.

The initial global quality of the product integrated all the descriptors generated. The initial overall quality was very good (9.8 out of 10), and decreased to approximately a value of 5 at the end of storage.

4. Conclusions

During storage at 45, 50 and 55 °C, slight changes ($p \leq 0.05$) in the pH values were registered, without impotence from a practical point of view. At the end of storage for each of the temperatures, there was a decrease in luminosity and an increase in the values of a* and b*, which corresponded to a browning of the product. The moment of sensory rejection, due to a notable loss of pineapple color and intense browning, coincided with the increase ($p \leq 0.05$) of the browning index.

Understanding changes in quality indicators, such as browning index and colorimetric coordinates, would be of interest so that producers of isotonic sports drinks can adjust storage conditions to maximize the product shelf life without compromising its quality. The study suggests that the loss of pineapple color is an important factor in the deterioration of the drink. These results could lead to further research into improving the formulation to reduce this effect, possibly by adding antioxidants or other stabilizing ingredients.

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