



The shelf life of standardized sugarcane juice stored under refrigeration

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Abstract

The shelf life (SL) of freshly extracted sugarcane juice is limited to a few hours, and the use of hurdle technology is a strategy to ensure its safety and stability. The SL of standardized cane juice (19.4 °Brix, 0.085% acidity and pH 4.35), pasteurized at 95 °C/30 s and stored under refrigeration, was estimated. Ideal (4 °C), commercial (8 °C) and abusive (12 °C) conditions were tested. Microbiological and sensory assays were carried out on the newly processed juice (time zero) and overtime to estimate the SL. Counts of psychrotrophic bacteria and molds and yeasts equivalent to 4 and 3 logCFU mL⁻¹, respectively, were set as acceptable maximum levels to establish the SL. Average scores greater than 5 (in the 9-point hedonic scale) and percentages of approval greater than 60% were both used as threshold values for juice's appearance and flavor. The juice's SL stored at 4, 8 and 12 °C were 94, 74 and 26 days, respectively. The combination of multiple technologies applied in this study was effective in obtaining a product with high sensory acceptance and a SL compatible with possible demands of the consumer market.

Keywords: hurdle technology; ultra clean filling; stability.

Practical Application: Combination of preservation methods to extend the shelf life of cane juice.

1 Introduction

Sugarcane juice is a highly acceptable drink in countries such as Brazil, India and China. However, the unprocessed juice offers perfect conditions for the growth of microorganisms and enzymatic reactions, which dramatically limits its shelf life (Yusof et al., 2000; Mao et al., 2007). The browning reactions that contribute to the deterioration of juice are triggered by both enzymatic (polyphenol oxidase and peroxidase) and nonenzymatic mechanisms (*Maillard* reaction, caramelization, thermal degradation reactions and condensation of sugars) (Bucheli & Robson, 1994). Moreover, the impurities such as gums, waxes, ash, coloring substances and soil contribute to shorten the juice's shelf life (Panigrahi et al., 2018).

In this scenario, the application of preservation technologies is required if the juice is to be bottled and stored. Shelf stability is especially problematic as the juice becomes contaminated with microorganisms. This presents obstacles to its industrial-scale production because it limits the manufacturer's opportunities. (Ramachandran et al., 2017). Considering the growing interest in the ready-to-drink cane juice, the development of processing technologies which promote security and stability, enable better distribution, and encourage the agroindustry is highly relevant.

The cane juice is characterized as an opaque liquid, brownish to dark green, with low acidity (pH 5.0-5.5), high water activity (0.99) and variable composition, depending on its variety, stage of maturity and planting conditions (Sankhla et al., 2012). Constituents of cane juice are grouped into three categories: (1) high molecular weight solutes (proteins, polysaccharides), (2) sucrose and (3) low molecular weight solutes (Panigrahi et al., 2018).

A well established and widely used concept in food processing is the hurdle technology. Some conventional methods for food

preservation, such as acidification with organic acids, heat treatment, aseptic and ultra clean filling, and refrigerated storage are applied in a "soft" but synergistic way (Wolti-Chanes et al., 2000) and are able to extend the shelf life.

The criteria to define the shelf life rely on the increase or decrease in the magnitude of the average value of a product characteristic. Sensory and microbiological methods are useful tools for this purpose (Hough, 2010). This study addressed the processing and shelf life evaluation of standardized cane juice stored under ideal, commercial and abuse refrigeration temperatures.

2 Materials and methods

2.1 Juice processing

Extraction

Sugarcane (*Saccharum officinarum*) cultivar SP 813250 was supplied by Tecnocana Tecnologia em Cana (Santa Cruz das Palmeiras, SP, Brazil). The juice was extracted and processed in a pilot plant located approximately 30 km from the cultivation site. Cane was cut, scraped and then immersed in a sodium hypochlorite (NaClO) solution containing 50 mg L⁻¹ free residual chlorine for 30 min at approximately 25 °C. Subsequently, the juice was extracted in a stainless-steel electric cylinder mill (Maqtron, Joaçaba/SC/Brazil).

Standardization

The juice was standardized by using a Central Composite Rotational Design (CCRD). The soluble solids content of 19.5 °Brix and acidity of 0.085% with a pH of 4.35, and a high sensory

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acceptance were achieved. Citric acid (as solid) and mineral water were both added to standardize the ratio (soluble solids/titratable acidity), as described by Geremias-Andrade et al. (2014).

Pasteurization and ultra clean filling

The standardized juice was pasteurized in a plate heat exchanger (Sumá Indústria e Comércio Ltda, Campinas/SP/Brazil) composed of regeneration, heating, holding and cooling sections, with a nominal capacity of 300 L h⁻¹. The temperature and holding time (95 °C/30 s) were set according to Kunitake et al. (2014). The processed juice was cooled and pumped into an insulated stainless steel tank. Bottling was performed using a gravimetric filler (Polienva-Movitron, São Paulo/SP/Brazil) in an unidirectional airflow cabin (ISO class 5) (Veco, Campinas/SP/ Brazil). Polyethylene terephthalate (PET) bottles (250 mL) pigmented with titanium dioxide (TiO₂) were previously decontaminated by spraying peracetic acid solution (PAA) at 0.05%(v/v)/5 s/45 °C (Thech Desinfecção, São Paulo/SP/Brazil). Polypropylene (PP) screw caps were decontaminated by dipping in the same solution. A volume of approximately 50 L was processed and then divided into four parts, stored at 4, 8, 12 and -18 °C (control) in the dark. Figure 1 shows the flowchart of processing ran in a pilot plant.

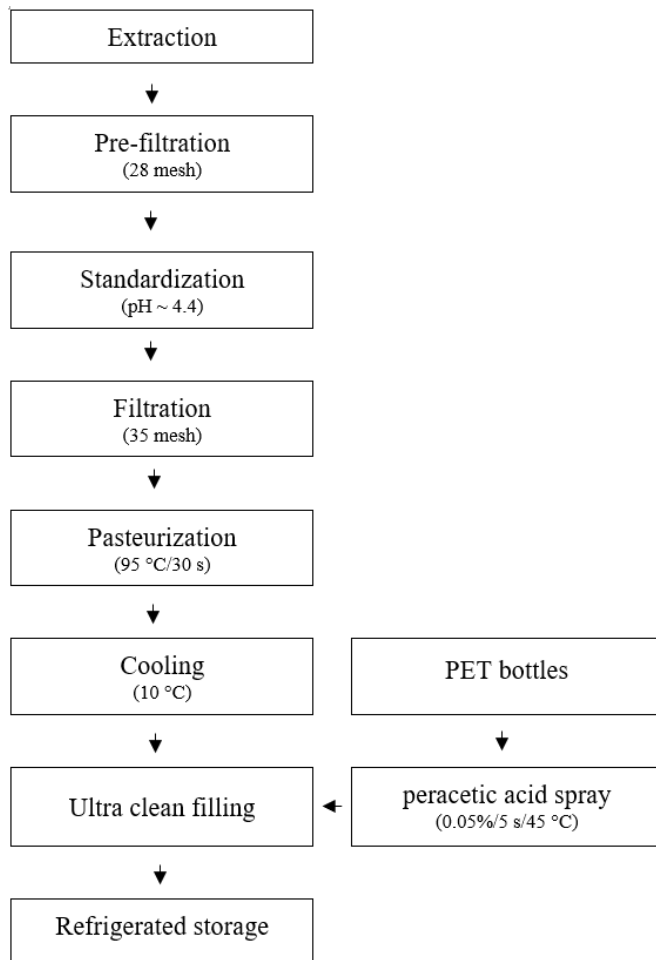


Figure 1. Flowchart of standardized cane juice processing.

2.2 Juice characterization

Physicochemical tests

Physicochemical tests were performed both before and after processing (Association of Official Analytical Chemists, 2012). To determine the pH, a pHmeter analyser Model 300 M (Hanna, Romania) was used. The soluble solids content was determined by a digital handheld refractometer Reichert Model AR 200 (Reichert, Depew/USA). The total acidity was determined using 0.1 M NaOH, this being the turning point where the pH value of 8.3 was obtained.

Enzyme activity

The protocols adapted from Campos et al. (1996) were used for measuring the polyphenol oxidase (PPO) and peroxidase (POD) activities.

Polyphenol oxidase

Seven milliliters of 0.2 M phosphate buffer solution (pH 5.5) and 1.0 mL of the diluted sample (juice) in deionized water (1:10) were added to a test tube and maintained in a heat bath at 35 °C 10 min⁻¹ to stabilize the temperature, then 1.5 mL of 0.05% guaiacol and 0.5 mL of 0.1% H₂O₂ were added. The tube was magnetically stirred for 15 s and returned to the heat bath at 35 °C 15 min⁻¹. Finally, the absorbance was read in a spectrophotometer at 425 nm. The blank was prepared by diluting the sample in deionized water.

Peroxidase

Five and half milliliters of 0.2 M phosphate buffer solution (pH 6.0) and 1.5 mL of 0.2 M catechol were added into a test tube and maintained at 25 °C 10 min⁻¹. Then 1.0 mL of the diluted sample in deionized water (1:10) was added. The tube was stirred for 15 s and returned to the water bath at 25 °C/ 30 min. The reading of absorbance was taken in a spectrophotometer at 470 nm. The blank was prepared by diluting the sample in deionized water.

One (1) unit of enzyme activity (U) was defined as the amount of enzymatic extract capable of increasing absorbance at 425 and 470 nm for PPO and POD, respectively, at rates of 0.001 unit per minute.

Microbiological assays

Counts of aerobic mesophiles and psychrotrophs were conducted according to the methodology described in the Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association (2001). Analyses of coliforms and *Salmonella* spp. were carried out to ensure the sanitary quality of the end product. To verify the presence of *Salmonella*, the rapid BAX System method by Du Pont was used. The results are qualitative (presence/absence) and the procedures were performed according to the manufacturer's guidelines. Analysis for coliform bacteria was performed using the 3M Petrifilm™ for quantification of *E. coli* and coliforms, as directed by the manufacturer.

2.3 Shelf life study

Microbiological assays

Microbiological assays for estimating the product's shelf life relied on psychrotrophs and molds and yeasts counts. Respectively, counts of 4 and 3 logCFU mL⁻¹ were set as the maximum acceptable levels. The frequency of analysis varied as a function of the storage temperature.

Sensory tests

A panel of 100 assessors (age of 22 years on average) was asked to evaluate the appearance and flavor of juice by assigning a liking score on a 9-point hedonic scale (Meilgaard et al., 2006). Samples were monadically presented in 50 mL plastic cups labeled with a 3-digit code and presented at a temperature of approximately 10 °C. One juice sample, obtained from the same production batch and stored at -18 °C, naturally thawed in refrigerator 24 h before the sensory analysis began, was taken to serve as control. The average scores greater than 5 and percentage of approval greater than 60% were set as threshold values for the attributes assessed. This study was approved by the Ethics in Research Committee from FZEA/USP (Report N 22450/ CAAE: 02584612.6.0000.5422).

2.4 Data analysis

The data were subjected to analysis of variance (ANOVA) and Tukey test, with 95% of confidence, using the software Statistical Analysis System (SAS 9.2).

3 Results and discussion

3.1 Physicochemical tests

Table 1 shows the physicochemical parameters of cane juice. To achieve the soluble solids content (~19.5 °Brix) and titratable acidity (~0.09%), as determined in the study of cane juice standardization conducted by Geremias-Andrade et al. (2014), mineral drinking water and citric acid were added to the raw juice just before pasteurization.

The pH determined in this study (5.55) was close to the range reported by Kunitake et al. (2013) (5.1 - 5.4), who used the same cultivar (SP 813250). In regard to yield, the author obtained values between 48 and 59%. Khare et al. (2012) obtained a yield of 52%. Both values were lower than that determined in this work (65%).

Table 1. Physicochemical parameters of cane juice (SP 813250).

	Raw juice	Standardized and pasteurized juice
pH	5.55 ± 0.00	4.35 ± 0.00
Soluble solids (°Brix)	20.7 ± 0.1	19.4 ± 0.0
Titratable acidity (% citric acid)	0.035 ± 0.001	0.085 ± 0.001
Polyphenol oxidase activity (U)	5.6 ± 1.0	0.7 ± 0.3
Peroxidase activity (U)	3.4 ± 0.9	1.6 ± 0.4

Yield in juice extraction (in relation to peeled raw material): 65% (w w⁻¹).

Kunitake et al. (2013) reported a wide variation in soluble solids content (15.2 - 23.6 °Brix) and titratable acidity (0.038 - 0.071 g citric acid 100 mL⁻¹) in freshly extracted juice from the same cultivar (SP 813250) and growth site.

As for the PPO and POD, the residual activity after heat treatment may have been influenced by the presence of isoenzymes. Robinson (2001) states the POD inactivation in plant-based foods is not linear because of the deviations in the kinetic reactions of the first order. This relies on the presence of a mixture of labile and thermostable isoperoxidases.

Studies carried out by Murasaki-Aliberti et al. (2009) addressed the PPO and POD inactivation in coconut water. The residual activity suggested the presence of two isoenzymes with different heat resistances. The log-reduction times at 87 °C ($D_{87\text{ }^{\circ}\text{C}}$) for PPO were 6 s and 11.3 min for labile and heat-resistant fractions, respectively. As for the POD, $D_{87\text{ }^{\circ}\text{C}}$ values were 8.6 s for the labile fraction and 26.3 min for the resistant one. In this study, the percentages of PPO and POD reduction were 88 and 53%, respectively. The heat treatment applied in this study (95 °C/30 s) was not totally effective in both PPO and POD inactivation. Mao et al. (2007) reported the PPO inactivation in acidified cane juice extracted from previously bleached raw material.

3.2 Microbiological assays

Raw juice

The average counts of aerobic mesophiles in raw juice were 3.9 logCFU mL⁻¹. The raw material naturally contains a high microbial population in their stems, roots and leaves, which is widely variable (Kitoko et al., 2004). The colonies of bacteria and molds and yeasts in the stems may range from 1 to 8 logCFU g⁻¹ and 1 to 3 logCFU g⁻¹, respectively (Duncan & Colmer, 1964). Therefore the counts in this study achieved acceptable levels.

Freshly processed juice

Neither coliforms at 45 °C nor *Salmonella* spp. were found in the end product. Brazilian regulation does not set limits for psychrotrophs and molds and yeasts counts in cane juice. Nevertheless, the Brazilian Health Regulation (RDC 12, 2001) set standards for pasteurized and refrigerated cane juice. According to that, the coliforms count at 45 °C must not exceed 10 CFU mL⁻¹ and *Salmonella* cells must be absent in 25 mL of product. Thus the results obtained in this study assure the product's safety.

3.3 Study of shelf life

The results hereby described were obtained from the standardized and pasteurized juice filled into PET bottles and stored under refrigeration in the dark.

Microbiological assays

As previously mentioned, psychrotroph bacteria and molds and yeasts (fungi) counts equivalent to 4 and 3 CFU mL⁻¹, respectively, were set as maximum acceptable levels.

With respect to samples stored at 4 °C, the counts were lower than 4 and 3 logCFU mL⁻¹ for psychrotrophs and fungi, respectively, after 94 days of storage. This finding indicates a good microbiological stability. The hurdles which favored the stabilization of juice were the acid pH, resulting from the addition of citric acid in the standardization step, the pasteurization, the aseptic bottling and the refrigerated storage.

Regarding the samples at 8 °C, the counts were lower than the pre-set maximum acceptable levels after 77 days of storage.

The counts in samples stored at 12 °C were lower than maximum acceptable levels during 32 days. That is an abusive temperature for food products which must be kept under refrigeration.

The microbiological assays were stopped as the sensory quality failed. The outcomes demonstrated a good performance from the processing and packaging technologies employed in the study. Chauhan et al. (2002) reported the increase in microbial population in acidified and pasteurized cane juice stored at 4 °C during 90 days. The counts ranged from < 1 to 2.1 logCFU mL⁻¹ for aerobic mesophiles, and from < 1 to 1.4 logCFU mL⁻¹ for yeasts and molds. In a study conducted by Yasmin et al. (2010), the juice was acidified to a pH of 4.3 and then pasteurized at 90 °C 5 min⁻¹. The product was hot filled into glass bottles and stored at 25 °C for 120 days. Over this time, there was no outgrowth of aerobic mesophiles and molds and yeasts.

Sensory tests

Sensory tests in this study proved to be a decisive factor in determining the product's shelf life. The sensory stability was determined by setting average scores greater than 5 in a 9-point hedonic scale and percentages of approval greater than 60% as threshold values. The samples were not served to assessors (panelists) every time any type of deterioration sign was noticed.

Storage at 4 °C

The evolution of the average scores for appearance and flavor of samples stored at 4 °C is depicted in Figure 2.

As shown in Figure 2, the juice survived until 94 days. The average scores and the percentages of acceptance were greater than 5 and 60%, respectively. The plots show a slight variation in the juice's acceptability over time. Additionally, the results obtained for sample (stored at 4 °C) and control (stored at -18 °C) were very close.

Storage at 8 °C

Figure 3 shows the evolution of the average scores obtained from samples stored at 8 °C.

Similarly to the juice stored at 4 °C, the samples at 8 °C shown a slight variation in terms of the appearance's acceptance. Still, the responses for sample (stored at 8 °C) were close to those for control (-18 °C). As for the flavor, the greater variation over time may be explained by the use of untrained assessors. Notwithstanding, the product survived until 74 days.

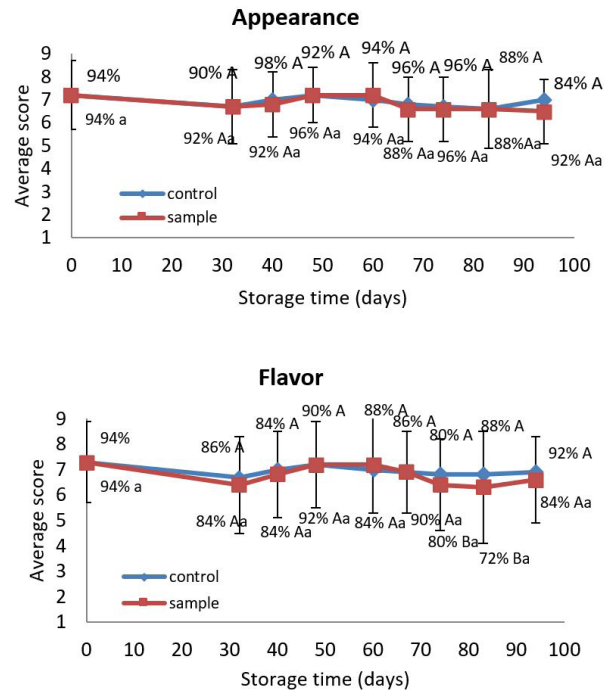


Figure 2. Average scores and percentage of acceptance obtained on the 9-point hedonic scale tests for samples stored at 4 °C. Averages with the same uppercase letter (comparison between control and sample) and with the same lowercase letter (comparison among storage times) are not different ($p > 0.05$). Vertical bars refer to standard deviation.

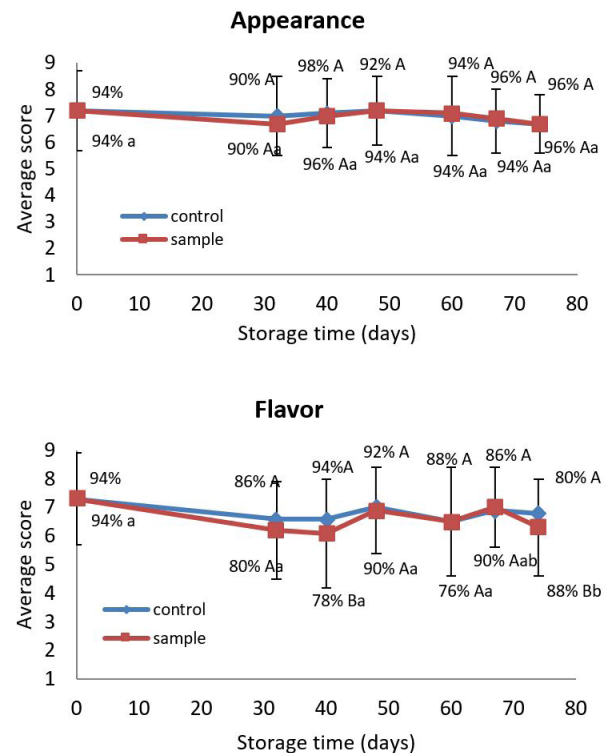


Figure 3. Average scores and percentages of acceptance obtained on the 9-point hedonic scale tests for samples stored at 8 °C. Averages with the same uppercase letter (comparison between control and sample) and with the same lowercase letter (comparison among storage times) are not different ($p > 0.05$). Vertical bars refer to standard deviation.

Storage at 12 °C

Figure 4 shows the variation on average scores over time for juice stored at 12 °C.

The increase of storage temperature to an abusive range dramatically shortened the sensory stability. The juice failed after 32 days; the percentage of acceptance for flavor was less than 60%. With respect to appearance, the plots show a very slight variation in the juice's acceptability over time. Moreover, the results obtained for the sample (stored at 4 °C) and control (stored at -18 °C) were very close. In regard to flavor, the greater variation over time may be explained by the use of untrained assessors. As far as the hedonic scale tests, this behavior is acceptable.

Because the failure time for the sample stored at 4 °C was observed at day 32, the best that can be said about the product

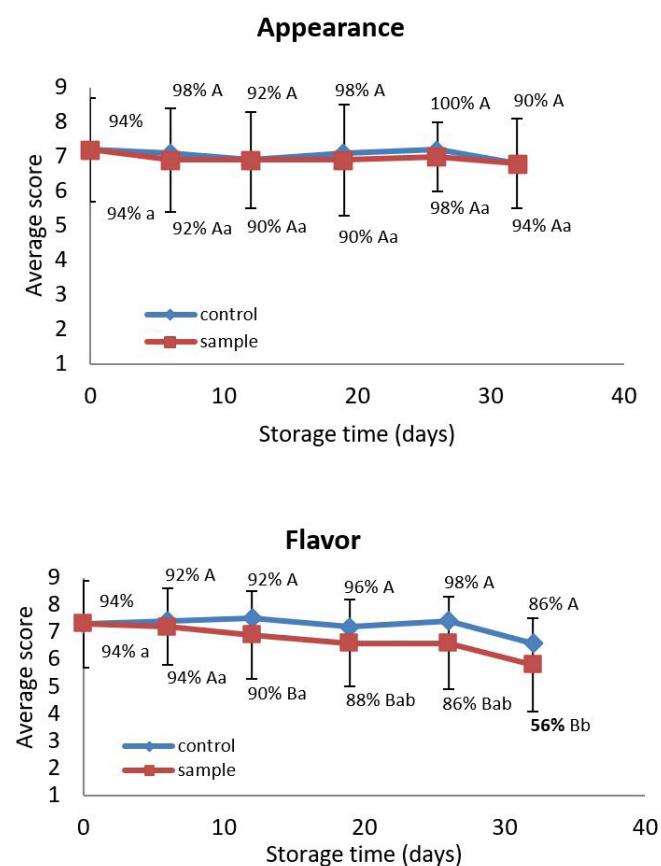


Figure 4. Average scores and percentage of acceptance obtained on the 9-point hedonic scale tests for samples stored at 12 °C. Averages with the same uppercase letter (comparison between control and sample) and with the same lowercase letter (comparison among storage times) are not different ($p > 0.05$). Vertical bars refer to standard deviation.

is that it survived for up to 26 days. According to Guillet & Rodrigues (2010), this situation, termed *interval-censoring*, is very common in shelf life testing because of the non-continuous monitoring of samples. In this event, it is virtually impossible to know the exact failure time of each sample. Thus the exact failure time for this sample is said to be interval-censored between 26 and 32.

The percentage of juice's acceptance at time zero (t_0) was very high (94%), for both appearance and flavor. Rates of approval above 70% show a good acceptance, according to Dutcoski (2013). In a study conducted by Silva & Faria (2006), the cane juice was treated at 141 °C 10 s^{-1} and then aseptically filled in sterilized glass bottles. In a second trial, juice was pasteurized at 110 °C 10 s^{-1} and then hot filled ($90 \pm 5 \text{ °C}$) into glass bottles. The findings showed that batches aseptically and hot filled achieved a shelf life of 30 and 60 days, respectively. Rezzadori et al. (2013) studied the effects of microfiltration and pasteurization at 90 °C 30 s^{-1} (P1) and 95 °C 30 s^{-1} (P2) on passion fruit pulp-added cane juice. A significant difference of 5% was found between the samples processed in P1 and P2 in terms of appearance, aroma, flavor and overall impression. The samples of P2 achieved higher scores (between "liked" and "liked very much") for appearance, flavor and overall impression; the percentage of approval was about 90%. However, the micro filtered product showed a better sensory quality when compared to thermally the one treated.

Physicochemical evaluation

Table 2 shows the variations in physicochemical parameters during the juice's shelf life.

In regard to pH, the greater variation was observed in the juice stored at 8 °C for 74 days; the pH decreased. With respect to soluble solids, there was no variation during the shelf life. As for the acidity, the greater variation occurred in the sample stored at 4 °C for 94 days; the acidity increased. However, one may consider that the small variations in pH and acidity (most likely related to the growth of microorganisms) did not have a meaningful effect on the sensory quality of juice, as suggested by Figures 2-4.

Cane juice's shelf life

The results of sensory and microbiological stability evaluations, in compliance with the pre-set criteria, were used to estimate the cane juice's shelf life. Table 3 shows the shelf life for product stored under different temperatures.

The results in Table 3 suggests a negative linear correlation ($R^2 = 0.95$) between the storage temperature, in the range of 4 to 12 °C, and the shelf life. Of particular relevance is the claim that the increase at the storage temperature from 8 to 12 °C had a

Table 2. Physicochemical variations in standardized and pasteurized cane juice stored under refrigeration during the product's shelf life.

	4 °C (94 days)	8 °C (74 days)	12 °C (26 days)
pH	- 0.17	- 0.19	- 0.09
Soluble solids (°Brix)	0.0	0.0	0.0
Titrateable acidity (% citric acid)	+ 0.059	+ 0.021	+ 0.011

Table 3. Shelf life of standardized and pasteurized cane juice stored under refrigeration in the dark.

Storage temperature (°C)	Microbiological stability (days)	Sensory stability (days)	Shelf life (days)
4	94	94	94
8	77	74	74
12	32	26	26

greater impact upon the juice's shelf life. Still, the microbiological and sensory stabilities were the same for the juice sample stored at 4 °C. Regarding the samples stored at 8 and 12, they were close to each other. Notably, the lower the temperature, under refrigeration conditions, the higher the storage cost. Depending on the intended shelf life to meet specific consumer market demand, the storage of the cane juice at 8 °C (74 day-shelf life) may be more strategic than storage at 4 °C (94 day-shelf life) in terms of cost saving. Kunitake et al. (2013) studied the effect of pasteurization temperature on the shelf life of passion fruit pulp-added cane juice stored at 7 °C. The juice's shelf lives treated at 85, 90 and 95 °C 30 s⁻¹ were 31, 39 and 52 days, respectively. As presented in Table 2, in this study the product's shelf life (74 days) stored at a close temperature (8 °C) was meaningfully longer than those reported by Kunitake.

Khare et al. (2012) processed cane juice to slow the deterioration rate and increase its shelf life. The authors used different combinations of potassium metabisulfite (150, 175, 200 and 225 mg L⁻¹), lemon juice (2.5-3.0%), sodium chloride (1.0-1.5%) and ginger extract (0.5-0.7%). Different pasteurization temperatures were tested (60, 75 and 90 °C 10 min⁻¹), and the product was stored under refrigeration. A shelf life of up to 60 days was achieved.

In a work conducted by Sankhla et al. (2012), the combination of pasteurization, antioxidants, acidulants and irradiation resulted in a juice with a shelf life of 60 days under ambient temperature (25 °C) and 90 days under refrigeration.

Ramachandran et al. (2017) added natural preservatives to cane juice aimed at extending its shelf life. Extracts of moringa, lemon and ginger were incorporated into raw and pasteurized (72 °C 15 s⁻¹) juice, stored at 2 and 6 °C. The optimum conditions were obtained by adding 10% extracts in pasteurized juice stored at 2 °C for 8 days. A combination of natural preservatives and low temperature storage was reported as an effective way of preservation for more than a month.

Garud et al. (2018) reported the combined effect of nonthermal hurdles (ozone and lactic acid) on cane juice's shelf life that was stored under refrigeration. They were comparable to heat treatment in maintaining microbial and sensory quality of product during 1-month storage.

4 Conclusions

The combination of processing and ultra clean filling technologies which was applied to cane juice in this study proved to be promising for micro-scale production, and also efficient in regard to the reduction of microbial contamination. The combined hurdles controlled the polyphenol oxidase and peroxidase activities to a moderate level. A beverage with a good sensory acceptance was achieved. The processed juice remained

microbiologically stable in lower temperatures throughout the study. The temperatures of 4 and 8 °C proved to be effective in maintaining an acceptable level of quality for a compatible period with possible demands of the consumer market. Finally, the outcomes shown the storage temperature played a meaningful impact upon the cane juice's shelf life.

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