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Sugarcane Juice Processing: Microbiological Monitoring

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Abstract

This study was undertaken to evaluate a pilot plant designed for sugarcane juice processing. The juice was extracted in an electric mill and acidified with citric acid until the pH of 4.3. Next, it was pasteurized in a plate heat exchanger at 95°C/30 sec, cooled to 10°C before being filled into a plastic bottle and induction sealed. Product filling was performed in an ISO class 5 unidirectional air-flow cabins. Three batches of acidified sugarcane juice were produced. The qualities of the raw material, rinse water of the processing and filling line, packaging and end product were all microbiologically evaluated. Hedonic scale tests were used to evaluate the sensory acceptance of the product. The total mean counts in mesophilic culture of molds and yeasts from the natural, fresh sugarcane juice were (6.26 and 5.20) log CFU/mL, respectively. These mean counts, in both rinse water samples of the processing line and the bottles, were lower than 1 log CFU/mL. The mean counts of molds and yeasts in acidified and pasteurized sugarcane juice were (2.63 and lower than 1) log CFU/mL, respectively. The findings indicated that the procedures that were evaluated met standards for acidified sugarcane juice to be produced then stored under refrigeration.

Highlights

a. A pilot plant for sugarcane juice processing was microbiologically monitored.

b. The enumeration of microorganisms showed low levels of contamination.

c. The stages of processing are targets for a commercial micro-scale production.

Keywords: Critical control points; Aseptic filling; Food sanitation

Introduction

The demand for the production of safe high quality food, which has both the sensory and nutritional characteristics similar to the raw material used and extended shelf life, is ever growing in the national market. However, some products, such as sugarcane juice, which is largely consumed in an informal marketplace, are frequently offered and sold in hygienic and sanitary conditions that are precarious at best. This presents a threat to the health of consumers. A clear example of this potential danger is the 2005 incident in the Brazilian state of Santa Catarina where sugarcane juice contaminated with the *Trypanossoma cruzi,* an etiological agent for Barber Bug fever (Chagas), was sold and publically ingested.

Sugarcane juice is a low acidity drink (pH>4.6) with a high water activity (Aw=0.99) and composed of approximately 80% water and 20% total dissolved solids. Among these solids, one may highlight saccharose (17%), glucose (0.4%) and fructose (0.2%), as well as nitrogenous substances such as organic acids and also minerals and such as iron, calcium, potassium, sodium and magnesium [1,2]. Spoilage microorganisms are the main contaminants responsible for the alteration of sugarcane juice; they are the primary cause of chemical, physical and sensory deterioration of the drink. Microbiological spoilage may be accelerated when abusive storage conditions create significant variations in the pH. Bacterial, molds and yeasts metabolize carbohydrates into acids and gums [3,4]. The advantages gained by the implementation of rational technologies to process sugarcane juice are related to the attainment of a safe drink of high quality that is available any time of the year, independent of the harvest season. Rational technologies extend shelf life, which in turn extend the area of distribution from plantation to consumer and also decrease transportation costs throughout the supply chain, especially in the volume of raw materials transported. In this scenario, the hurdle technology (combined processing) is a concept widely employed in the food processing industry. Examples of hurdles active in stabilizing sugar cane juice include the acidification (pH<4.6), thermal treatment, the aseptic filling of packaging preciously decontaminated and the refrigerated storage of the end product. In a food processing system, it can be understood as Control Point (CP) whereby any step or procedure regarding biological, chemical or physical factors can be controlled, primarily by prerequisite programs such as Good Manufacturing Practices (GMPs) and Sanitarian Standard Operating Procedures (SSOP). This concept differs from the Critical Control Points (CCP), which is controlled by the Hazard Analysis and Critical Control Points (HACCP) system. A CCP can be any step or procedure in which preventive measures to control an identified hazard are applied in order to eliminate, prevent or reduce the health risk of the consumer [5-7]. This study encompassed the microbiological monitoring of a pilot plant designed for the processing of sugarcane juice aseptically filled into plastic bottles. Additionally, standard procedures of operational hygiene were implemented to produce a drink that is safe and maintains an elevated sensory quality.

Material and Methods

Sugarcane juice extraction

The raw material (*Saccharum officinarum*, cultivar SP 81-3250) was provided (previously peeled) by Tecnocana Tecnologia em Cana Ltda, Santa Cruz das Palmeiras/SP/Brazil and processed 24 hours after harvest. After the sanitation of the sugarcane by immersion in a solution of 5% (m/v) sodium dichloroisocyanurate ($C_3Cl_2N_3NaO_3$), at 25°C for 20 minutes, the sugarcane extraction was effected using an electric mill

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made of stainless steel cylinders manufactured by Maqtron, Joaçaba/SC/Brazil.

Standardization of pH

After filtering the bagasse residue, the sugarcane juice had its standard pH established at a value equal to 4.3. To this end, citric acid was added to the juice, thus creating an acidified drink.

Heat treatment

The pasteurization of the standardized sugarcane juice was effected by using a plate heat exchanger equipped with a return pneumatic valve for the unpasteurized product, manufactured by Sumá Indústria e Comércio Ltda, Campinas/SP–Brazil. After the heat treatment of 95°C/30s, the drink was cooled to approximately 10°C and then transferred to an airtight insulated container, where it remained for 1 hour before packaging.

Packaging asepsis

The bottles were decontaminated by spray washing a peracetic acid solution 0.05% (v/v) at 45°C for 20 Sec. The spraying system was acquired through the Casa das Cantinas, Bento Gonçalves/RS - Brazil. Polypropylene (PP) caps with aluminum seals were sterilized at 121°C/15 min.

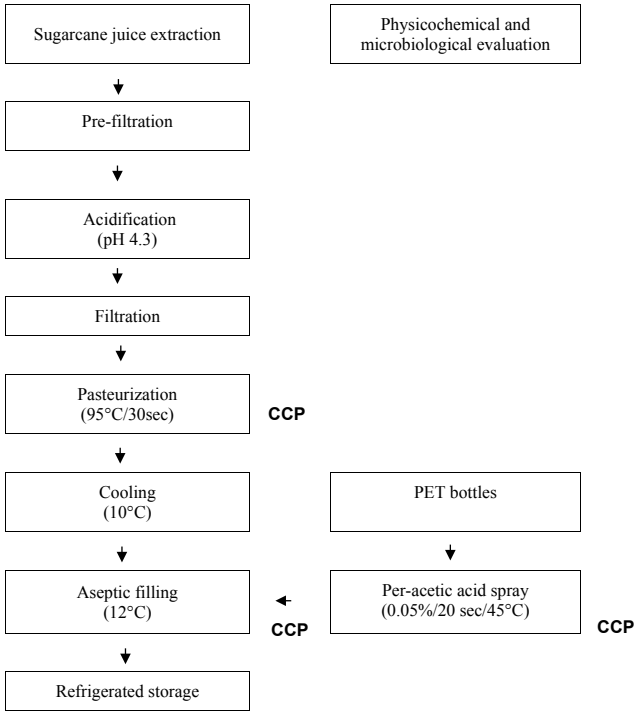

Figure 1: Flowchart of experimental production of sugarcane juice.

Aseptic bottling

The aseptic filling was performed in a horizontal unidirectional airflow cabin (ISO class 5) made of stainless steel, manufactured by Veco do Brasil, Campinas/SP Brazil. For this purpose, semi-automatic gravimetric filler was employed, manufactured by Polienva-Movitron, São Paulo/SP–Brazil. The filling was done into white pigmented PET bottles with a volume capacity of 330 mL, hermetically sealed with polypropylene screw lids (PP) with aluminum seals by electromagnetic induction and using a sealer manufactured by Enercon Industries Corporation, model Super Seal Jr. The packaging was acquired from Plasvipack Importação e Exportação Ltda, São Paulo/SP–Brazil.

Processing

Figure 1 illustrates the flowchart stages taken in the processing of standardization and pasteurization of the sugarcane juice. Three batches of the drink were produced. The highlighted operations represent the hurdles (extrinsic factors) which favor the microbiological stabilization of the drink. The critical control points are identified as CCP. Figure 2 depicts both the raw material processed in this study and the end product.

Sanitization of the processing line

The alkaline cleaning of the processing line was carried out with a solution of alkaline detergent Sandet® 874, with 0.0067% (67 ppm) of NaOH, at 85°C during 20 min. After the rinse to remove any alkaline residue, the acid cleaning was followed with a solution of acid detergent Sandet® 162, with 0.0025% (25 ppm) of HNO_3, at 70°C during 20 min and then followed by a final rinse. The cleaning (in-place) was performed immediately after processing. The sanitation was immediately performed before processing with a peracetic acid (PAA) solution Peroxide® P170 in concentration of 0.1% (v/v) at 50°C, during 40 min.

Determination of soluble solids content and pH values

The determinations of soluble solids content and pH values were performed in order to characterize the raw material. Measurements of pH were also carried out to check the final pH of the product after the acidification. In the determination of the pH, a pH measurer from MS Tecnopon, model mPA 210 (Piracicaba, São Paulo, Brazil) was used. For the determination of soluble solids content, a digital refractometer from Reichert AR 200 (Depew, New York, USA) was used.

Figure 2: (a) Raw material, (b) Aseptic bottling and (c) Pasteurized sugarcane juice.

Microbiological monitoring

The microbiological analysis of samples collected in different stages of the processing was carried out in compliance with the methodology described by Silva et al. [8]. The microbiological quality of the raw material, the water used in the pilot plant, the water used in the final rinse of the processing line and filling, the enumeration of contaminants present in the packages before and after the decontamination processing and the end product were all evaluated. The counting of sporulated microorganisms was not performed because this group was not used as a target of the processing technologies applied in this study after the sugarcane juice was acidified (pH 4.3), pasteurized and stored under refrigeration.

Analysis of natural fresh (non-pasteurized) sugarcane juice: In each processing, three samples of 50 mL of sugarcane juice were sampled after the extraction process. The samples of the fresh (natural) juice were submitted to the total counting of aerobic mesophilics in Plate Count Agar (PCA) and molds and yeasts in Potato Dextrose Agar with chloramphenicol (PDA-c), in accordance with the methodology described in the Compendium of Methods for the Microbiological Examination of Foods, APHA [9].

Rinse water of processing line: Three samples of 200 mL rinse water (pasteurized and cooled) were collected immediately following the cooling section of the heat exchanger and in the nozzle of the aseptic filler. The samples of rinse water were vacuum filtered through a cellulose membrane with porosity of 0.45 μm. The membranes were transferred to petri dishes that also contained PDA and PDA-chloramphenicol, incubated at 35°C and 25°C, respectively.

Packages: The microbiological analysis of the bottles was conducted separately and distinctly from the lids and seals. Before decontamination, the bottles were divided in three batches containing six units each, making a total of 18 units. The same separation process was made after the decontamination of the bottles. The procedure adopted in the analysis of the bottles consisted of the addition of 50 mL of sterilized solution into the packages which contained 0.94% (v/v) of surface-active Tween 80; 0.50% (v/v) of a sodium thiosulfate solution of 10% (m/v) and 98.56% of a peptone water solution of 0.1% (m/v). After the bottles were closed, they were vigorously shaken in the standardized manner. Samples of rinse water used in the packages were vacuum filtered through a cellulose membrane with porosity of 0.45 μm. The membranes were transferred into petri dishes containing PDA and PDA-chloramphenicol(c), incubated at 35°C and 25°C, respectively. The results were expressed in CFU per bottle. The analysis of the lids and aluminum seals before and after sterilization (121°C/15 min) was accomplished by twisting a moistened (in 1 mL of peptone water 0.1%) sterile cotton swab, this being then transferred to a 9 mL test tube of peptone water, which was stirred in Vortex for 5 minutes. An aliquot of 0.1 mL was transferred to a PDA plate and an aliquot with the same volume was transferred to a PDA-c. All samples were analyzed for aerobic mesophilic microorganisms counts (incubated at 35°C/96 h) and were enumerated for molds and yeasts in PDA-c (25°C/96 h). The results were expressed in CFU by lid/seal.

End product: To assess the number of decimal reductions in the microorganism population reached by pasteurization, the end product samples were analyzed concerning the total number of aerobic mesophilic and molds and yeasts, in accordance with the methodology described in the Compendium of Methods for the Microbiological Examination of Foods, APHA [9].

Sensory evaluation

Hedonic scale tests were used to evaluate the acceptance of the product based on sensory results of a team consisting of 102 panelists (age of 22 years on average) of sugarcane juice. This study was approved by ethics committee of University of Sao Paulo n. 631.732. The panelists were asked to evaluate the sensory attributes of appearance and flavor by assigning a liking score on a 7-point hedonic scale (1=disliked very much; 4=neither liked nor disliked; 7=liked very much). Tests were accomplished in individual booths lighted with a white fluorescent lamp, and the samples were monadically presented in 50 mL plastic cups labeled with a 3-digit code and presented at a temperature of about 10°C. Mineral water was provided to cleanse the palate.

Statistical analysis

The data statistical tests relied on the analysis of variance (ANOVA) and on Tukey's test which was used for the means comparison. For this purpose, the SAS program (SAS Institute, Inc., Cary, NC, USA), version 9.2, was used.

Results and Discussion

The three performed batches differed from each other in regard to the mass of processed raw material, the volume of sugarcane juice extracted and the number of packages produced, as shown in Table 1.

Table 1 show that the average yield obtained in the extraction of sugarcane juice was of 50%. Mao et al. [10] once obtained the average yield of extracted sugarcane juice from bleached sugarcane close to 71% value that showed it to be superior to the one obtained in this present study. The difference between both results may be related to the juice extraction method. Higher levels are usually obtained in industrial scales and also may occur because of the enzymatic treatment of the bagasse. On the other hand, Khare et al. [11] obtained an extraction yield of 52%, close to values determined in this study. Despite the increasing variation in the mass of processed raw material and the volume of the extracted juice, the number of packages produced did not follow the same proportion. This fact is due to the loss of the drink volumes, which were variable, during the processing and the filling of the end product.

Determination of soluble solids content and pH values

Table 2 shows the average values of pH and soluble solids content determined in the fresh and natural (non-pasteurized) sugarcane juice. Results with similar pH were obtained by Gallo and Canhos [12], with

Batch	Raw material[1] (Kg)	Volume of sugarcane juice extracted (L)	Extraction yield (%)	Number of packages
B1	82.61	41.0	50	80
B2	96.63	49.0	51	107
B3	103.10	53.5	52	81
[1]previously peeled.				

Table 1: Mass of processed raw material, volume of extracted sugarcane juice and number of packages produced.

Processing	pH	Soluble solids (°Brix)
P1	$5.11^{a} \pm 0.01$	$20.4^{c} \pm 0.1$
P2	$5.07^{ab} \pm 0.02$	$23.7^{a} \pm 0.1$
P3	$5.03^{b} \pm 0.02$	$22.8^{b} \pm 0.1$
Means (three replicates) with the same exponent, in the same column, are not different (p>0.05).		

Table 2: Mean values of pH and soluble solids determined in freshly extracted sugarcane juice.

values between 5.0 and 5.5. Considering the soluble solids content, Oliveira et al. [13] related an average of 22.74 ± 1.4 °Brix, a very similar value to the one found in this work. Rezzadori [14] obtained a value slightly inferior, equal to 19.4 °Brix. In Table 2 it can be observed that, although the sugarcane juice pH of P1 and P3 differ from each other, the subsequent acidification allowed the patronization of the product; in doing so, the importance of this step in the processing line was evident. The differences observed among the soluble solids content may be due to different periods of sugarcane maturation and times of year influenced by periods of drought.

Microbiological monitoring

Natural fresh (non-pasteurized) sugarcane juice: The averages obtained in the counting of aerobic mesophilic, molds and yeast in the recently extracted sugarcane juice (non-pasteurized) was equivalent to (6.26 and 5.20) log CFU/mL, respectively.

The current Brazilian Food Legislation (Resolution RDC n°12 on 2 of January of 2001 by ANVISA [6]) does not establish any standard for the total counting of aerobic mesophilic, molds and yeasts in sugarcane juice.

Research conducted by Gandra et al. [15] and Prati [16] indicated that the total aerobic mesophilic counting of (6 and 7) log CFU/mL may trigger the occurrence of objectionable sensory changes, culminating in the deterioration of the product. Silva et al. [8] reported that molds and yeasts are commonly found in non-pasteurized sugarcane juice. Oliveira et al. [13] found some counting equivalent to 6.15 log CFU/mL, a log cycle superior to the average value (5.20 log CFU/mL) obtained in this study. In this context, the averages counting determined in this research, suggest the importance of an immediate appliance of technologies that inhibit or destroy the initial contaminant population of the sugarcane juice, in order to reach an extended shelf life.

Rinse water of the processing line: Table 3 shows the averages on the microorganisms counting in the final rinse water (heated to 95°C/30 s) of the processing and filling line after sanitation. It´s noteworthy that initial counts for both total mesophilic and molds and yeasts were close to 10^4 CFU/mL. The data presented in Table 3 show that the averages of mesophilics and molds and yeasts in the final rinse water of the processing and filling line were substantially reduced, displaying the effectiveness of the sanitation procedures implemented.

Packages: Table 4 shows the results of the microbiological analysis of the PET bottles before and after their decontamination by spray washing peracetic acid (PAA) with 0.05% (v/v) at 45°C for 20 sec. With the exception of the average obtained in the counting of bottles used in the processing 2, before decontamination, the results of Table 4 show that the initial contamination of the packages was extremely low. This fact made it difficult to assess the performance of the PAA for the intended purpose, because the average counting of the packages

Processing	Processing line[1]		Filling point[2]	
	Total mesophilic	Molds and yeasts	Total mesophilic	Molds and yeasts
P1	$<1.0_{est}$	$<1.0_{est}$	5.0	$<1.0_{est}$
P2	34	$<1.0_{est}$	35	4.7
P3	9.3	2.0	34	14

[1]Sampling in the pasteurizer output.
[2]Sampling off the metering.
est - estimated values. Average of three samples.

Table 3: Averages (CFU/100 mL) of total aerobic mesophilic and molds and yeasts counting in the final rinse water.

(CFU/bottle)				
Processing	Before		After	
	Total aerobic mesophilics	Molds and yeasts	Total aerobic mesophilics	Molds and yeasts
P1	<1	<1	<1	<1
P2	1.3×10^2	<1	<1	<1
P3	<1	<1	<1	<1
Average of 18 bottles.				

Table 4: Microbial counting before and after decontamination of the bottles.

(CFU/lid-seal set)				
Processing	Before		After	
	Total aerobic mesophilics	Molds and yeasts	Total aerobic mesophilics	Molds and yeasts
P1	<1	2.2	<1	<1
P2	1.1	<1	<1	<1
P3	1.7	<1	<1	<1
Average of 18 samples analyzed before and after decontamination.				

Table 5: Microbial counting before and after the lids and seals set sterilization.

(estimated) before and after decontamination was lower to 1.0 CFU/bottle.

Similar results were found in the sterilized lids and seals, as is shown in Table 5. It is noteworthy that the procedure for package decontamination was identified as a CCP, involving a microbiological hazard, because it represents a potential risk of the product´s recontamination. If a recontamination occurs there is no further step to control an eventual failure, after the product is packed and after its pasteurization.

End product: The averages obtained in the aerobic mesophilic counting and the molds and yeasts counting in the processed drink were equivalent to 4.3 × 102 (2.63 log CFU/mL) and<1.0 CFU/mL, respectively. Prati et al. [17] reported an aerobic mesophilic counting equivalent to 2.95 log CFU/mL for the sugarcane juice, acidified, clarified and pasteurized at 75°C/15 sec and packed in PET bottles. This is a similar value to the one determined in this study. In respect to molds and yeasts, the counting obtained by Prati et al. [17] was of 1.71 log CFU/mL, which is an average very close to the one found by Oliveira et al. [13], who pasteurized the acidified sugarcane juice at 70°C /25 min that had previously been filled into high density polyethylene bottles (HDPE).

In another study, Silva and Faria [18] obtained aerobic mesophilic counting and the molds and yeasts counting lower at 1.0 CFU/mL in acidified and processed sugarcane juice at 141°C/10 s that had been aseptically and "hot filled" into glass bottles. Tables 6 and 7 compare the averages found in microbial counting in sugarcane juice before and after its processing. According to data from Table 6, the averages obtained in the counting of non-processed samples of P2 and P3 were two log cycles higher than the average obtained in P1. This fact demonstrates the lack of uniformity in the microbial contamination of the sugarcane. In contrast with the processed product, no statistical difference was observed among the counting results on all three samples of processing.

Another fact worth highlighting is that the number of decimal reductions reached by thermal processing was equivalent to 2.7; 3.4 and 3.9; for P1, P2 and P3, respectively. Due to the sugarcane juice acidification to a pH value of 4.3 and subsequent treatment at 95°C/30 s, a higher number of decimal reductions was expected in this study [19]. With respect to molds and yeasts, no statistical difference was observed among the samples from the three processes. Molds and yeasts colonies

Processing	Total aerobic mesophilics counting (logCFU/mL)	
	natural/nonpasteurized	end product
P1	4.3^{Ab}	1.6^{Ba}
P2	6.3^{Aa}	2.9^{Ba}
P3	6.5^{Aa}	2.6^{Ba}
Means (three replicates) followed by the same uppercase exponent in the same row (comparison between natural and processed juices), and means with the same lowercase exponent in the same column (comparison among processes) are not different (p>0.05).		

Table 6: Average counting of aerobic mesophilics in the sugarcane juice.

Processing	Molds and yeasts counting (log CFU/mL)	
	natural/non-pasteurized	end product
P1	4.43^{a}	<1
P2	4.11^{a}	<1
P3	5.65^{a}	<1
Means followed by the same exponent are not different (p>0.05).		

Table 7: Molds and yeasts counting in sugarcane juice.

Batch	(Average ± standard deviation/% acceptance[1])		
	Appearance	Aroma	Flavor
B1	5.84^{a} ± 1.24/84	5.91^{a} ± 1.20/85	6.04^{a} ± 1.24/86
B2	6.08^{a} ± 1.11/87	6.14^{a} ± 1.00/88	5.96^{a} ± 1.21/85
B3	5.86^{a} ± 1.27/84	6.05^{a} ± 1.02/86	5.88^{a} ± 1.32/84
Means followed by the same exponent, in the same column, are not different (P>0.05) regarding the product´s acceptance. [1]Percentage of panelists that assigned scores above 4 using a 7-point hedonic scale: (1=disliked very much; 4=neither like/nor dislike; 7=liked very much).			

Table 8: Average scores from the 7-point hedonic scale tests obtained from the three batches of the acidified (pH 4.3) and pasteurized sugarcane juice.

were not found in the end product samples. In this way the results were presented as estimated values (<1 log CFU/mL). It is noteworthy that the numbers of decimal reductions achieved by pasteurization were higher to 4.4; 4.1 and 5.7; for P1, P2 and P3, respectively. Finally, the results of Tables 6 and 7 showed that the processing technologies used in this, the most current study, were more effective in the destruction of molds and yeasts when compared to the total aerobic mesophilics.

Sensory evaluation

The average scores obtained from the hedonic scale tests that evaluate the acceptance of the sugarcane juice are presented in Table 8. The results presented in Table 8 reveal the high acceptance of the juice, because the average scores for the three attributes (appearance, aroma and flavor) were greater than 5.8 for the three batches performed. Similarly, the acceptance percentages have been very high, ranging between 84 and 88%. Such results demonstrate the good sensory quality achieved by the processed sugarcane juice. Finally, it's worth mentioning that the means were not statistically different from each other. This suggests uniformity of the sensory quality of the end product.

Conclusion

The enumeration of microorganisms at the processing and filling line, in the packages and in the end product showed low levels of contamination. Furthermore, a beverage with a high sensory acceptance was achieved. Microbiological monitoring in the pilot plant demonstrated that the stages of production were efficient targets for an eventual micro-scale production of sugarcane juice, which is acidified, pasteurized then stored under refrigeration.

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