academicJournals

Vol. 7(31), pp. 3992-3995, 2 August, 2013
DOI: 10.5897/AJMR2013.5854
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Changes in the microbial population of pasteurized soursop juice treated with benzoate and lime during storage

Nwachukwu, E.* and Ezeigbo, C. G.

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Accepted 22 July, 2013

The microbial quality of locally produced soursop juice and the effect of pasteurization, acidification and chemical preservative on the juice were investigated. Soursop juice samples were analyzed according to standard bacteriological and chemical methods. Microbiological quality of the treated juice (pasteurized at 60°C for 1 h, acidified with lime and addition of 0.05% sodium benzoate) was evaluated. Microbial load increased in the sample (without treatments- control), from 4.3×10^6 to 14.6×10^6 cfu ml^{-1}. Pasteurized soursop juice showed a decrease in microbial load from 2.2×10^3 to 4.2×10^1 cfu ml^{-1}. Juice treated with sodium benzoate also decreased in microbial load from 1.2×10^6 to 2.3×10^5 cfu ml^{-1} while acidified soursop juice decreased from 2.3×10^6 to 4.8×10^3 cfu ml^{-1}. Pasteurized soursop juice increased in pH from 4.89 to 4.98 and a decrease in titrable acidity from 23.25 to 21.92. Soursop juice treated with sodium benzoate increased in pH from 5.94 to 6.23 and a decrease in titrable acidity from 23.62 to 18.10. Acidified soursop juice decreased in pH from 4.03 to 3.95 and an increased but with steady titrable acidity of 37.50. The study reveals that the synergistic effect of pasteurization, acidification and treatment with sodium benzoate can be used to extend the shelf-life of soursop juice for up to two weeks.

Key words: Soursop juice, microbial quality, pasteurization, acidification, benzoate.

INTRODUCTION

Fruit juices are important sources of nutrients and contain several important therapeutic properties that may reduce the risk of various diseases. They contain large amounts of antioxidants, vitamins C and E, and possess pleasant taste and aroma (Abbo et al., 2006).

Juices produced from tropical fruits have increasingly gained global importance due to their health effect. There are different types of tropical fruits (e.g. orange, grape, pineapple, banana, guava and watermelon) readily available for the production of fruit juices. The juice may be produced from single fruit or combination of fruits and sold by the street vendors. Meanwhile, there are other underutilized tropical fruits such as soursop that have not been used for the processing of fruit juices.

Soursop (*Annona muricata* L.) is a juicy fruit, commonly found in the Southern part of Nigeria, and mostly eaten as fresh fruit (Abbo et al., 2006). However, soursop can become a potential source of raw material for juice drink production. The juice can be extracted and packaged for human consumption. Freshly processed soursop juice drink can be consumed immediately or stored in the refrigerator or treated with preservatives. Freshly expressed juice is subject to rapid microbial growth, enzymatic or chemical and physical deterioration. Therefore, any possible way of processing that will minimize these undesirable reactions and enhance the inherent quality of the starting fruit should be encouraged.

Benzoic acids and its salts are among the most

*Corresponding author. Email: drejik@yahoo.com.

commonly used antimicrobial agents for improving storage ability of fruit juices. Their wide usage is due to their broad-spectrum activity against yeasts, moulds and bacteria, as well as their non-alteration of food flavor (Fleet, 2003). However, pasteurization is one of the most commonly used techniques aimed at reducing the microbial load (Umme et al., 2001; Larissa et al., 2011). The aim of this study was to investigate the optimal conditions required for extending the shelf-life of soursop juice. The objectives were to ascertain:

1. The chemical quality changes,
2. The microbiological quality of pasteurized and unpasteurized soursop juice,
3. The microbiological quality of pasteurized soursop juice treated with sodium benzoate, and
4. The effect of lime or acidification on soursop juice.

MATERIALS AND METHODS

Source of samples

Ten (10) fresh and fully ripe soursop (*A. muricata* L.) were obtained from Umudike market in Umuahia, Nigeria, packaged in sterilized polyethylene bags and analysed within 2 h.

Preparation of samples

The fruit was rinsed in warm distilled water, surface sterilized with 70% ethanol, hand peeled and deseeded (sterile gloves worn). Hundred grams (100 g) of the pulp was blended with 900 ml of sterile distilled water inside a blender (Moulinex, type 278, China). The fruit was homogenized, filtered (through muslin cloth) to obtain fruit juice. Then, 15 ml portion from the juice was dispensed into each of 30 sterile bottle containers.

Treatment of juice

Chemical preservative: Five (5) containers that have pure juice, served as the control. Then, 0.05% (w/v) sodium benzoate (Sigma chemical company) was aseptically added to another set of five containers. All the ten containers were stored at ambient temperature (30 - 31°C) for two weeks.

Pasteurization

Five containers of pure juice were transferred into a hot water bath and pasteurized at 60°C for 1 h (Maji et al., 2011). The product was then removed, cooled and stored at ambient temperature (30 to 31°C) for the period of the experiment. Another group of five containers were pasteurized and 0.05% (w/v) sodium benzoate was aseptically added and stored at ambient temperature for a period of two weeks.

Acidification

Soursop juice was acidified to pH 4.03 with lime fruits. Lime fruits were first surface-sterilized (70% ethanol) and peeled using a pre-sterilized knife. The fruits were then halved (using a pre-sterilized knife) and the juice was squeezed aseptically (sterile gloves worn during preparation) into sterile 100 ml conical flasks. To five other containers, 2 ml of lime juice with pH 3.62 was added. The remaining five containers having the pure juice were pasteurized at 60°C for 1 h, and 2 ml of lime juice was added aseptically.

pH determination

The pH of each group of containers with the different treatment was determined using a pH meter (Jenway pH meter, Model 3310) for the period of storage (8 days/times), at two days interval from the first day. The pH of each of the treated juice samples and the control was measured thrice and recorded.

Total microbial count

Nutrient agar was prepared based on the manufacturers' instruction, and sterilized in an autoclave for 121°C for 15 min. Each experiment was carried out in duplicates. Serial dilutions of the juice were carried out and 0.1 ml (using sterilized 1ml pipettes) of the appropriate aliquot was then added unto the agar plate. Different sterile 1 ml pipette for individual treatments was employed. Spread plate technique was adopted and the cultured plates were incubated at 37°C for 48 h. Thereafter, the average of colonies formed on duplicate plates for each treatment was recorded. The total microbial count was recorded as colony forming units per milliliter (cfu ml^{-1}).

Titrable acidity

Ten (10) ml of soursop juice was measured into a 250 ml conical flask, and four drops of phenolphthalein indicator was added. This was titrated with the standard 0.1 N NaOH to faint pink point. The titer for total acidity was expressed as g citric acid/L (Umme et al., 2001).

Data analysis

The data was subjected to analysis of variance (ANOVA) and the means were separated using pre-packaged computer statistical software (SPSS 17.0).

RESULTS

The effect of different treatments on the microbial load of the soursop juice throughout the period of storage is shown in Table 1. Microbial load was reduced in the pasteurized juice from 2.2×10^3 to 4.2×10^1 cfu ml^{-1}, with a significant difference at $p<0.05$. Juice treated with sodium benzoate recorded a decrease from 1.2×10^6 to 2.3×10^5 cfu ml^{-1}, while the acidified samples decreased in the microbial load from 2.3×10^6 to 4.8×10^3 cfu ml^{-1}. The result revealed a steady decrease in the microbial load of pasteurized soursop juice treated with acidification and chemical preservative.

Table 2 shows the effect of the different treatments on the pH of soursop juice. pH of the acidified juice decreased significantly ($P<0.05$) from 4.03 to 3.95. There was a significant increase ($p<0.05$) in pH for juice samples with benzoates from 5.94 to 6.23. Pasteurized juice increased in pH from 4.89 to 4.98. Juice treated with pasteurization and acidification had a slight increase in

Table 1. Effects of different treatments on the microbial population of soursop juice during the period of storage.

Treatment	Days of storage				
	0	2	4	6	8
Pure juice (control)	$4.3×10^{6a}$	$7.8×10^{6b}$	$14.6×10^{6b}$	$19.4×10^{6b}$	$28.6×10^{6b}$
Pure juice + lime	$2.3×10^{6b}$	$3.7×10^{4b}$	$4.8×10^{3b}$	$8.2×10^{3b}$	$9.1×10^{3b}$
Pure juice + benzoates	$1.2×10^{6b}$	$1.7×10^{5b}$	$1.4×10^{5b}$	$3.9×10^{5b}$	$5.8×10^{5b}$
Pure juice + pasteurization	$2.2×10^{3b}$	$2.1×10^{2b}$	$3.8×10^{2b}$	$5.1×10^{2b}$	$7.2×10^{2b}$
Pure juice + pasteurization+ lime	$2.0×10^{6b}$	$1.2×10^{6b}$	$3.4×10^{4b}$	$5.6×10^{4b}$	$8.4×10^{4b}$
Pure + pasteurization + benzoates	$2.1×10^{4b}$	$1.8×10^{3b}$	$1.4×10^{3b}$	$3.8×10^{3b}$	$7.1×10^{3b}$

Values are means of duplicate determinations. Values within a column with the same superscripts are significantly different (p<0.05).

Table 2. Effect of different treatment on the pH of soursop juice during the period of storage.

Treatment	Days of storage				
	0	2	4	6	8
Pure juice (control)	4.82^b	4.92^b	4.78^a	4.94^b	4.62^a
Pure juice +lime	4.03^a	4.07^a	3.99^a	4.15^b	3.95^a
Pure juice + benzoates	5.94^a	6.05^b	6.21^b	6.18^b	6.23^b
Pure juice + pasteurization	4.89^b	4.92^b	4.94^b	4.95^b	4.98^b
Pure juice + pasteurization + lime	3.32^a	3.46^a	4.05^b	4.07^b	4.27^a
Pure juice + pasteurization + benzoates	6.05^b	6.10^b	6.20^b	6.23^b	6.26^b

Values are means of duplicate determinations. Values within a column with the same superscripts are significantly different (p<0.05).

pH from 3.32 to 4.27. Treatment with pasteurization and sodium benzoate increased in pH from 6.05 to 6.28.

Table 3 shows the effect of the varying treatments on the titrable acidity of soursop juice over the period of storage. The titrable acidity for acidified samples remained stable at 37.50 for the period of storage and recorded no significant increase (p<0.05). Titrable acidity for samples treated with benzoates decreased from 23.62 to 18.10. Pasteurized juice samples decreased in titrable acidity from 23.25 to 21.92. A similar trend was observed in samples treated with both pasteurization and benzoates which decreased from 24.37 to 20.20 with a significant difference (p<0.05).

DISCUSSION

The microbial quality of pasteurized soursop juice locally produced and the effect of acidification with lime and chemical preservative on the juice were investigated. The effects of the varying treatments were investigated with a view to prolong the shelf-life of soursop juice. Biodeterioration of the fruit is influenced by factors like temperature, pH, chemical composition and microbial load.

The results of this investigation reveal that untreated soursop juice (control) recorded a significant increase (p<0.05) in microbial load during the period of storage. This could probably be due to the microbial activities favored by the absence of refrigeration and presservatives. Treatment of soursop juice with lime juice showed a decrease in microbial load. This finding therefore suggests that the addition of lime juice to soursop juice might prolong the shelf-life of the juice. The reduction of the bacterial load in soursop juice following the addition of lime juice is the acidic nature of the juice. The excellent keeping quality of fruits and soft drinks is influenced by low pH (Bates et al., 2001). This is because low pH tends to inhibit bacterial growth. Therefore, the addition of lime should be encouraged since it can inhibit bacterial growth in soursop juice. Moreover, lime fruits are not hazardous and hence safe for human consumption.

Meanwhile, antimicrobials can effectively extend the shelf-life of fruit products (Ukwo et al., 2010). The use of 0.05% sodium benzoate produced a decrease in microbial load indicating that it could be used for preserving unrefrigerated juice for at least two weeks. Benzoates are preferable in the preservation of fruit juices due to the solubility of their salts. Benzoates are used in low temperatures to extend the shelf-life of minimally processed juices (Uma et al., 2011). Their

Table 3. Effects of different treatments on the titrable acidity of soursop juice during the period of storage

Treatment	Days of storage				
	0	2	4	6	8
Pure juice (control)	26.10[a]	25.50[a]	24.30[a]	22.10[b]	20.37[a]
Pure juice +lime	37.50[b]	37.50[b]	37.50[b]	37.50[b]	37.50[b]
Pure juice + benzoates	23.62[a]	22.59[a]	21.82[a]	19.22[b]	18.10[a]
Pure juice + pasteurization	23.25[a]	23.05[a]	22.91[b]	22.50[b]	21.92[a]
Pure juice + pasteurization + lime	37.50[b]	37.50[b]	37.50[b]	37.50[b]	37.50[b]
Pure juice +pasteurization +benzoates	24.37[a]	24.22[a]	22.84[b]	21.98[b]	20.20[a]

Values are means of duplicate determinations. Values within a column with the same superscripts are significantly different (p<0.05).

wide usage is due to their broad-spectrum activity against some microorganisms, as well as their non alteration of food flavor.

Moreso, pasteurized soursop juice recorded a decrease in microbial load. This decrease could be that pasteurization of the fruit juice destroy pathogenic and spoilage organisms. Pasteurized juice treated with lime and benzoates resulted in a decrease in microbial load. This may possibly be due to the synergistic effect of pasteurization, acidification and treatment with chemical preservative.

The control sample recorded a significant ($p<0.05$) decrease in the pH of soursop juice during the period of storage. This could probably be as a result of the activities of inherent microorganisms leading to fermentation and production of organic acids such as acetic acid. An increase in acidity could also be as a result of the presence of microbial metabolites over time. Acidified juice significantly ($p<0.05$) decreased in pH. The use of lime juice for this study was simply due to the acidic nature of lime and most microorganisms do not strive in acidic conditions. The option of acidifying the soursop juice with lime juice, rich in citric acid, is aimed to lower the pH of the juice, making the juice unfavourable for microbial growth. Acidified and pasteurized juice also recorded decrease in pH. This combination discourages most bacterial growth (Sodini et al., 2002; Lutchmedial et al., 2004). Acidity increases the efficiency of heat processing and inhibits the growth of surviving heat resistant microorganisms (Frazier and Westhoff, 1998).

However, a significant ($p<0.05$) increase in the pH of juice treated with sodium benzoate as well as in pasteurized juice was observed. Decrease in titrable acidity corresponded to the increase in pH.

The findings of this study generally indicated that combination of acidification, pasteurization and chemical preservation is suitable for preservation of soursop juice for some days without refrigeration. These methods considerably decreased the microbial count. Since these methods are simple, inexpensive and convenient, they can be adopted for industrial use in the processing and preservation of soursop juice. The utilization of the preserved juice should be encouraged as health/therapeutic drink. Above all, preservation of soursop juice is important because of the seasonality of its production which makes it abundantly available during its season and scarce during off season. Therefore, low cost preservation of soursop juice using pasteurization alone, or preferably a combination of pasteurization and sodium benzoate, as well as acidification with lime juice is recommended for extending the shelf-life of the juice stored at ambient temperature.

REFERENCES

Abbo ES, Olurin T, Odeyemi G (2006). Studies on the storage stability of soursop (*Annona muricata* L.) juice. Afr. J. Biotechnol. 5:108-112.

Bates RP, Morris JR, Crandell PG (2001). Principles and practices of small and medium scale fruit juice processing. Food agricultural services, United Nations, Rome.

Fleet GH (2003). Yeast interactions and wine flavour. Int. J. Food Microbiol. 86:11-22.

Frazier WC, Westhoff DC (1998). Food microbiology (4th Ed). New Delhi: MacGraw-Hill. p. 196.

Larissa MR, Geraldo AM, Maria LG, Paulo HM, Evania AF (2011). Effect of heat treatment on microbiological properties of mixed tropical fruits nectars. J. Food Technol. (9):124-128.

Lutchmedial M, Ramlal R, Badrie N, Chang-Yen I (2004). Nutritional and sensory quality of stirred soursop (Annona muricata L.) yoghurt. Int. J. Food Sci. and Nutri. 55:407-414.

Maji AA, Omale J, Okoli EC (2011). Effects of chemical treatment and pasteurization on the shelf life of kunun zaki (sorghum and maize gruel). European J. Food Res. and Rev. 1(2):61-70.

Sodini I, Lucas A, Oliveira MN, Remeuf F, Coirre G (2002). Effects of milk base and starter culture on acidification, texture and probiotic cell counts in dalam fermented milk processing. J. Dairy Sci. 85:2479-2488.

Ukwo SP, Ezeama CF, Ndaeyo NU (2010). Growth of different yeast strain during fermentation of soursop (*Annona muricata*) juice as influenced by acetic acid bacteria. Nature Sci. 8(10):285-291.

Uma T, Rama RV, Khasim BS (2011). Preservation and shelf life extension of cashew apple juice. Internet J. Food Safety 13:275-280.

Umme A, Bambang SS, Salmah Y, Jamilah B (2001). Effect of pasteurisation on sensory quality of natural soursop puree under different storage conditions. Food Chem. 75:293-301.