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Microbiological Assessment of Preservative Methods for African Star Apple (*Chrysophyllum albidum Linn*) Juice

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Abstract: Microbiological assessment of preservative methods for African Star Apple juice was carried out. The juice samples were subjected to pasteurization, chemical treatment using 0.1% (v/v) sodium benzoate, a combined treatment of both or no treatment. All samples were stored in ambient (28±2°C) or refrigeration (5°C) temperatures for six weeks to determine the effect of treatments on growth count. The juice sample that was pasteurized and preserved with sodium benzoate stored longer than any other sample at both storage conditions. The combination of pasteurization, use of sodium benzoate and juice storage at refrigeration temperature gave the best storage stability.

Key words: African star apple juice, sodium benzoate, juice storage

INTRODUCTION

Post harvest loss of fruits can be minimized by processing of fruits into products like juices, nectars and concentrates. In addition to providing longer stability, fruit processing can also be used to alter the natural or inherent nutrients through fortification, thereby making it richer than the original fruit content, make the fruits readily available especially for the seasonal fruits and also more convenient to consume (Chukwumalume and Nnaji, 2010).

A study was carried out on the formulation of juice from African star apple by Chukwumalume *et al*. (2010). The results revealed that a nutritious fruit juice with low sugar content can be produced from the fruit. However, it is noteworthy that food items with high moisture content readily spoil or get decomposed by microorganisms. Moreover, merely processing a food from its natural form to another is not enough to prevent or minimize the effect of these spoilage microorganisms if special preservation techniques are not employed (Prescott *et al*., 2005). There are different methods in food preservation such as: use of high temperature, use of low temperature, drying, use of chemicals, irradiation, maintenance of anaerobic conditions, removal of microorganisms and asepsis (Basar and Rahman, 2007). Food preservation can involve one or more of the different methods.

This research assessed the efficacy of selected preservation techniques for African star apple juice-physical (pasteurization and refrigeration) and chemical (sodium benzoate) methods-using microbiological tests.

MATERIALS AND METHODS

Preparation of African star apple juice: The fresh star apple fruits were randomly purchased from retailers within Zuba fruit market in the Federal Capital Territory, Abuja. The work was based on simple randomized design. The fruits were sorted, washed, air dried, cut open, deseeded and the pulp scraped out, blended in a 1:1 fruit to distilled water ratio, filtered, dispensed into 200 ml sterile plastic containers, stored at -16°C (freezer) and taken from time to time for various analyses.

Preservation with sodium benzoate: Sodium benzoate, was prepared by the method of Edison and Kalama (2003). The test samples from the freezer were first equilibrated to room temperature and then formulated into 10 ml volumes; this was achieved by pipetting 1 ml of the sodium benzoate stock solution into 9 ml of the sample to be preserved.

Pasteurization: Pasteurization of the fruit juice sample was carried out by the method of Awan and Okaka (1985). The pasteurized samples were divided into two groups: one group was used to assess the efficacy of pasteurization whilst in the other group, 1 ml sodium benzoate solution was added to 9 ml juice (v/v) to assess the efficacy of a combined treatment of pasteurization and sodium benzoate.

Microbial analysis: Fresh sample of juice formulations were serially diluted 1:100 and plated from 10^{-2} using spread plate technique on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) selective media for mould and yeast as stated in Jideani and Jideani (2006).

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Also, the treated juice with preservative, pasteurized only and pasteurized with preservative were immediately withdrawn after treatment and subjected to similar analysis.

There were two sets of 4 samples viz: Fresh (untreated as control); Fresh + Preservative (0.1% sodium benzoate); Pasteurized only and Pasteurized with Preservative (0.1% sodium benzoate).

Sampling and counting of microbial growth on samples: The 4 juice samples were stored in different 10 ml volume plastic bottles totaling 96 for total aerobic counts plating and 56 bottles for total fungal counts all making a total of 152 bottles. One set, made up of 48 bottles for total aerobic count and 28 bottles for total fungal counts, was stored at room temperature (RMT: 28±2°C) and the second set was stored at refrigeration temperature (REF. 5°C). One bottle from each of the 4 samples and from each set was mixed thoroughly, serially diluted as 1:100 and spread plated in triplicate from 10^{-2}. This was repeated at interval of 4 and 7 days for total aerobic count (NA growth) and fungal counts (PDA growth) respectively.

Method of plating: Disposable sterile Petri dishes were used. The plates were dated and labeled. The spread plate technique as described by Bandler *et al.* (1998) and Downes and Ito (2001) were used. Plating was done in triplicates and the average determined.

The number of Colony Forming Unit (CFU) was counted per plate using a Gallenhamp colony counter. Total number of organisms per ml of sample was calculated thus:

CFU/ml = Average plate count x Dilution factor/volume of inoculums

Statistical analysis: The data obtained was statistically analyzed (Analysis of Variance) using Statistical Package for Social Scientists (SPSS version 13) by comparing the three treated samples with the control and within/between treatments under ambient and refrigerated storage conditions.

RESULTS AND DISCUSSION

Product quality: Chukwumalume *et al.* (2010) stated that the fruit juice which was cloudy, conformed to Codex description of fruit juice, processed as a single strength juice, that is, juice from one fruit source without additive (Codex, 2003). Sodium benzoate was used at 0.1% (v/v) as this is the permitted level for human consumption (Frazier and Westhoff, 1995; Codex, 2003) for highly acidic juice (pH 2.9) to avoid further acidification. There were no additives like sugar so as to avoid interference with the fruits' natural flavor especially the astringent characteristic.

According to Davis and Houghton (1987), there are several factors that can encourage or limit the growth of microorganisms in juices, these include: water activity, pH, hygiene practice, storage temperature and concentration of preservatives, these were well articulated in the course of producing this juice.

The juice was processed employing the principles of Good Manufacturing Practice (GMP): Hazard Analysis And Critical Control Points (HACCP) to ensure that sources of contamination were reduced to the barest minimum. This included washing of fruits before handling, gloves were worn throughout operation and distilled water was boiled before utilization.

Effects of treatments: The control sample stored under ambient condition had an aerobic count of 4.45±0.82 Log_{10} (2.80 x 10^4) cfu/ml by the 8 th day, which had exceeded the maximum recommended count (Table 4). At ambient temperature, the control storage showed highest aerobic counts of 5.18±0.21 Log_{10} (1.50 x 10^5) cfu/ml on the 12th day, an initial aerobic count of 3.43±0.18 Log_{10} (2.6 x 10^3) cfu/ml and the highest fungal counts of 5.12±1.48 Log_{10} (1.34 x 10^4) cfu/ml on the 7th day (Table 1a and 1b). The control sample however, lasted for 12 days under refrigerated condition before exceeding the recommended standard count.

Table 1a: Total aerobic counts for control stored at ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TAC (log_{10} cfu/ml)	Refrigerated Temp. (5°C) TAC (log_{10} cfu/ml)
0	3.43±0.18	3.43±0.18
4	3.90±0.60	3.58±0.40
8	4.45±0.82	3.74±0.56
12	5.18±0.24	3.96±0.55
16	5.09±0.70	4.45±0.18
20	4.99±1.18	4.56±0.72
24	4.82±1.36	4.60±1.22
28	3.95±0.40	4.67±0.30
32	3.07±0.24	4.70±65.0
38	2.82±0.24	4.76±0.30
40	2.42±0.24	4.81±1.00
44	2.22±0.00	4.88±1.04

Values are triplicate means±SD. TAC = Total Aerobic Counts

Table 1b: Total fungal counts for control stored at ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TFC (Log_{10} cfu/ml)	Refrigeration temp. (5°C) TFC (Log_{10} cfu/ml)
0	3.22±0.18	3.22±0.18
7	5.12±1.48	3.88±0.48
14	4.85±1.00	4.08±0.66
21	4.65±1.35	4.24±0.66
28	4.44±0.98	4.33±0.49
35	3.97±0.32	4.48±0.80
42	2.22±0.24	4.59±0.32

Values are triplicate means±SD. TFC = Total Fungal Counts

For the refrigerated samples there was a gradual rise in viability count both for aerobic and fungal (Table 1a, 1b, 2a, 2b, 2a, 3b, 5a and 5b), however, growth did not exceed 10^4 in all refrigerated samples.

Table 2a: Total aerobic counts for fresh juice treated with 0.1% sodium benzoate stored at ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TAC (Log_{10} cfu/ml)	Refrigerated Temp. (5°C) TAC (Log_{10} cfu/ml)
0	3.07±0.24	3.07±0.24
4	3.52±0.18	3.18±0.00
8	3.73±0.06	3.30±0.00
12	4.39±0.61	3.45±0.24
16	4.53±0.40	3.74±0.00
20	4.72±0.30	3.83±0.18
24	5.01±0.88	3.98±0.00
28	4.82±1.07	4.06±0.42
32	4.58±0.77	4.14±0.18
36	3.96±0.18	4.20±0.30
40	3.70±0.00	4.27±0.32
44	3.37±0.18	4.32±0.30

Values are triplicate means±SD. TAC = Total Aerobic Counts

Table 2b: Total fungal counts for fresh juice treated with 0.1% sodium benzoate stored

Time (Days)	Ambient storage (28±2°C) TFC (Log_{10} cfu/ml)	Refrigeration storage temp. (5°C) TFC (Log_{10} cfu/ml)
0	0.00	0.00
7	2.22±0.24	0.00
14	2.52±0.24	2.22±0.24
21	3.37±0.24	2.52±0.24
28	3.98±0.00	2.82±0.22
35	3.88±0.00	2.82±0.22
42	3.74±0.00	3.26±0.24

Values are triplicate means±SD. TFC = Total Fungal Counts

The unpasteurized fresh sample with preservative at ambient storage showed initial aerobic counts of 3.07±0.24 Log_{10} (1.2×10^3) cfu/ml, highest aerobic counts of 5.01±0.88 log_{10} (1.02×10^5) cfu/ml on the 24th day, highest fungal counts of 3.98±0.00 Log_{10} (9.5×10^3) cfu/ml on the 28th day (Table 2a and 2b).

The application of only pasteurization resulted in no microbial growth on the media for 4 days under ambient and 24 days under refrigerated. This is indicative of the effect of heat treatment on microorganisms (Jay, 1987), when compared with both the control and the fresh juice preserved with sodium benzoate samples (Table 1a-3b).

A comparison of the counts obtained under refrigerated storage with the recommended standard Table 4 (FDA, 1996), showed that the refrigerated pasteurized sample did not get spoilt for 6 weeks period of study. Conversely, the pasteurized sample under ambient storage got spoilt after three weeks as aerobic count of 4.40±0.70 Log_{10} (2.5×10^4) cfu/ml was recorded on the 28th day (Table 3a and 3b).

This conforms to Adams and Moss (1995) and Prescott *et al.* (2005) who affirmed that pasteurization kills only part and not all the microbes. Moreover, Frazier and Westhoff (1995) stated that storage at ambient temperature encourages proliferation of microorganism. This implies that with a combined treatment of pasteurization and refrigeration, the juice can store up to six weeks.

Table 3a: Total aerobic counts for pasteurized sample stored under ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TAC (Log_{10} cfu/ml)	Refrigerated Temp. (5°C) TAC (Log_{10} cfu/ml)
0	0.00	0.00
4	0.00	0.00
8	2.22±0.24	0.00
12	2.70±0.00	0.00
16	3.00±0.00	0.00
20	3.30±0.00	0.00
24	3.81±0.42	0.00
28	4.40±0.70	2.22±0.24
32	4.87±0.70	2.52±0.24
36	4.65±1.01	2.82±0.22
40	4.43±0.69	3.00±0.00
44	4.24±0.37	3.34±0.14

Values are triplicate means±SD. TAC = Total Aerobic Counts

Table 3b: Total fungal counts for pasteurized juice sample stored under ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TFC (Log_{10} cfu/ml)	Refrigerated Temp. (5°C) TFC (Log_{10} cfu/ml)
0	0.00	0.00
7	0.00	0.00
14	2.22±0.24	0.00
21	2.82±0.24	0.00
28	3.00±0.00	2.22±0.24
35	3.30±0.00	2.52±0.24
42	3.50±0.18	2.82±0.22

Values are triplicate means±SD. TFC = Total Fungal Counts

Table 4: Recommended microbiological standard for any fruit juice (FDA, 1996)

Count (cfu/ml)	Max count anticipated	Max count permitted
Total counts	5.0×10^3	1.0×10^4
Yeast	100	1.0×10^3

Storage conditions: Microorganisms increased faster in ambient condition than in refrigerated condition (Frazier and Westhoff, 1995) with the highest total aerobic count of 5.18±0.24 Log_{10} (1.50×10^5) cfu/ml recorded from the untreated (control) sample on the 12th day (Table 1a and 1b), which was obvious indication of spoilage as compared with recommended microbiological standard shown in Table 4 (FDA, 1996).

The proliferation of microorganisms under the ambient temperature was fast but with a subsequent sharp decline (Table 1a and 1b). This fall can be attributed to depletion of nutrients and production of inhibitory metabolites (Narang, 2004). While under the refrigerated storage condition, a relatively slower rate of proliferation was observed in initial low counts, with a steady gradual increase which conforms to Prescott *et al.* (2005).

Unpasteurized fresh sample with 0.1% v/v sodium benzoate showed slight difference from the control sample under both storage conditions as the juice deteriorated. This implies that the sodium benzoate was not effective in inhibiting the inherent organisms without pre-pasteurizing the juice. This agrees with Prescott *et al.* (2005), which established that food items will readily deteriorate due to microorganisms if special techniques like the use of high or low temperature are not employed in preservation.

Table 5a: Total aerobic counts for sample pasteurized and treated with sodium benzoate (0.1% v/v) and stored under ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TAC (Log_{10} cfu/ml)	Refrigerated Temp. (5°C) TAC (Log_{10} cfu/ml)
0	0.00	0.00
4	0.00	0.00
8	0.00	0.00
12	0.00	0.00
16	0.00	0.00
20	0.00	0.00
24	0.00	0.00
28	0.00	0.00
32	2.22±0.22	0.00
36	2.51±0.24	0.00
40	2.70±0.24	0.00
44	2.90±0.00	0.00

Values are triplicate means±SD. TAC = Total Aerobic Counts

Table 5b: Total fungal counts for sample pasteurized and preserved with sodium benzoate (0.1% v/v) and stored under ambient and refrigerated temperatures

Time (Days)	Ambient storage (28±2°C) TFC (Log_{10} cfu/ml)	Refrigerated Temp. (5°C) TFC (Log_{10} cfu/ml)
0	0.00	0.00
7	0.00	0.00
14	0.00	0.00
21	0.00	0.00
28	0.00	0.00
35	0.00	0.00
42	0.00	0.00

Values are triplicate means±SD. TFC = Total Fungal Counts

The combined treatment of pasteurization and 0.1% sodium benzoate (v/v) indicated good shelf stability. These samples stored longer than any other sample at both storage conditions. No aerobic count was recorded for this sample under refrigerated storage. There was no fungal count at both refrigerated and ambient storage for the 6 weeks period of study, however there was aerobic counts 2.20±0.20 Log_{10} (1.58×10^2) cfu/ml from this sample at ambient storage on 32nd day (Table 5a and 5b).

Nevertheless, for the period of study, the growth count under the ambient storage was not indicative of spoilage as the count was below the maximum permissible count recommended FDA (1996) (Table 3a, 3b, 5a and 5b).

Only the combined treatment of pasteurization and sodium benzoate showed effective suppression of contaminants (Frazier and Westhoff, 1995) as there was significant difference ($p<0.05$) at both ambient and refrigerated temperatures when compared with the control sample under similar storage conditions. This result showed that under low temperature of refrigeration storage of 5°C, the microbial activities were retarded in the sample with only chemical treatment, but growth was slower in pasteurized sample stored at the same temperature as this was indicative of the efficacy of the combined heat treatment of pasteurization and low temperature storage effect on the viability of microorganisms and conformed to Birch *et al.* (1998).

There was no significant difference between the control and unpasteurized sample with preservative and the control and pasteurized only sample under ambient storage (Table 1a, 1b, 2a and 2b). All three samples under ambient storage got spoilt before the end of the study except the sample pasteurized and preserved with sodium benzoate. This can be attributed to the claim by Narang (2004), that the microorganisms were denatured and lysed by pasteurization and the chemical preservative respectively.

Furthermore the pasteurized and preserved sample when stored at the refrigeration temperature did not spoil throughout the period of study. This is in conformity with Codex (2003) and Edison and Kalama (2003) on the combination of the use of pasteurization, chemical preservative and low temperature storage to ensure prolonged shelf stability of fruit juice. This could mean that the activities of the spoilage organisms are suppressed at this temperature.

Conclusion: The juice sample that was pasteurized and preserved with sodium benzoate stored longer than any other sample at both storage conditions. The combination of pasteurization, use of sodium benzoate and juice storage at refrigeration temperature gave the best storage stability.

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