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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effect of Sodium Benzoate and Storage Periods on Nutrient, Phytochemical and Organoleptic Attributes of Mature Unripe Pawpaw (*Carica papaya*) Fruit Juice

J.N. Chikwendu, O. Ugwuanyi and R.O. Edeh
Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract: This study determined the effect of preservative and storage periods on the nutrient, phytochemical and organoleptic attributes of mature unripe *Carica papaya* fruit juice. The juice was prepare and sodium benzoate was added to the juice as a preservative. The juice was pasteurized at 70-80°C for 4 min, filled in sterilized bottles, corked and stored at room temperature (25±2°C). The nutrient, phytochemical and organoleptic attributes of the juice were determined daily for two weeks using standard methods. The data was analyzed using Duncan's multiple range test to separate and compare the means. The effect of the preservative was less on the macronutrient composition. There was a significant reduction in the minerals and phytochemicals as storage duration increases. The preservative generally reduced the rate of nutrient loss in the juice when compared with other studies without preservatives.

Key words: Nutrient, phytochemical, organoleptic attributes, sodium benzoate, mature unripe pawpaw fruit juice

INTRODUCTION

In the tropics, fruits grow in abundance, in home gardens and in the wild. They are rich in micronutrients and phytochemical (Potter and Hotchkiss, 1997; Nakasone and Paul, 1998). Fruits, increase interest for diet consumption as well as variety, colour, taste and aroma (Potter and Hotchkiss, 1997; Nakasone and Paul, 1998). Food products undergo spoilage and deterioration during storage due to growth and activities of microorganisms, food enzymes and other chemical reactions within the food itself. The effect of infestation by pests, storage temperature, moisture, oxygen, light and physical stress or abuse (Potter and Hotchkiss, 1997) on seasonal and perishable agricultural products varies. When fruits are in season, they are in abundance and many are wasted due to poor storage. This poses a challenge to fruit producers. There is a growing trend towards adding values and varieties to raw agricultural products such as fruits (Nakasone and Paul, 1998). Preservatives are substances added to foods to extend their keeping quality and increase nutritive value (Marrian, 1998). Shelf life is a time food products remain safe, to retain desirable sensory, chemical, physical and microbiological characteristics. These foods must comply with any label declaration for nutritional potentials and storage under recommended conditions (IFST, 2000).

Many extrinsic and intrinsic factors operate and interact to inhibit or stimulate a number of processes to limit shelf life (IFST, 2000). This interaction inhibits or stimulates a number of processes to adversely affect shelf life. The shelf life of a food product depends on a number of factors such as processing method, preservative, packaging and storage condition. Shelf life is time within which a product remains stable (Potter and Hotchkiss, 1997). The chemical composition of commercial juice is dependent on the processing and storage conditions (Mears and Shenton, 1998). Consumers are increasingly interested in both nutrition and health benefit of foods. Consumers growing concerns are on basic nutritional benefits of food associated with disease prevention and health enhancing compounds contained in many foods (Hasler, 1998).

The objective of the study is to assess the effects of preservatives on nutrient, phytochemical and organoleptic attributes of mature unripe *Carica papaya* juice stored at various periods.

MATERIALS AND METHODS

Preparation and production of sample: The mature unripe pawpaw fruits (4.5 kg) were harvested from a specific pawpaw tree in a farmland to ensure homogeneity of the sample (fruits). The sample were sorted for wholesomeness, washed with clean water and salt to remove contaminants from the surface of the fruit. The washed pawpaw fruit (sample) were peeled and cut using sterile stainless knife. The fruits were grated to tiny particles using local grater. The juice was squeezed out with muslin cloth. Preservative (sodium benzoate 500 mg/L) was added to the juice, pasteurized at 70-80°C for 4 min, filled into sterile bottles, corked, stored at room temperature 25±2°C and was analyzed weekly for two weeks.

Corresponding Author: J.N. Chikwendu, Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria

Chemical analysis: The sample was subject to chemical analysis to determine the macro and micro nutrients composition as well as the phytochemicals. The proximate content of the juice at various storage periods were analyzed in triplicates. Fat composition was determined by solvent extraction; Protein was determined using micro Kjedahl method; moisture, ash and crude fibre were determined according to AOAC (2005) procedures and total carbohydrate was determined by difference (Pearson Chemical Analysis of Food, 1976).

Vitamin B_1 and ascorbic acid were determined as described by AOAC (2005). About 5 g sample was dissolved in distilled water, 2 ml of trichloro-acetate was added, colour was developed with 2, 6-dichloroindophenol. The colour was read with spectrophotometer (Model 3030 Perkin Elmer, Nortwalk, USA).

The samples were wet-digested with concentrated nitrate and perchlorate for mineral determination. Sodium (Na) and potassium (K) were determined by flame photometer AOAC (2005). Iron (Fe) and zinc (Zn) were determined by atomic absorption spectrophotometer (Model 3030 Perkin Elmer, Nortwalk, USA) (AOAC, 2005). Iodine (I) was determined as described by AOAC (2000) method. Flavonoids, tannins, saponins and terpenoids were determined using standard methods (Boham and Kocipai, 1974; Van-Burden and Robison, 1981; Obadin and Ochuko, 2001; Subhadhirakul and Pechpongs, 2005).

Sensory evaluation: The organoleptic characteristics were determined by a panel of 30 judges comprising of staff members and students of Home Science, Nutrition and Dietetics Department, University of Nigeria Nsukka, Enugu State, Nigeria. A-9 point hedonic scale were 9 represented the highest score and 1 the lowest was developed as an instrument for the sensory evaluation as described by Derek and Richard (Derek and Richard, 1982). The organoleptic attributes evaluated were colour, flavour, taste, mouth feel and general acceptability of the juice. The products were appropriately coded before presentation.

Statistical analysis: Data from the study were analyzed using Analysis of Variance (Steel and Torrie, 1960) and T-test. Means were separated using Least of significant difference and Duncan Multiple range test. Significance was accepted at 5% probability.

RESULTS AND DISCUSSION

Table 1 presents the effect of sodium benzoate and storage periods on proximate composition of mature unripe pawpaw (Carica papaya) fruit juice. The moisture value varied. The variation was from 90.64 to 93.01%. The W_0 sample had the least (90.64%) and W_2 sample had the highest value (93.01%). The W_0 and W_1 sample had 90.64 and 90.85%, each. The preservative and pasteurization reduced microflora degradation. Fruit juice had high moisture (Enwere, 1998). The high moisture value for the juice did not ensure storage stability regardless of low p^H (4.75 to 5.10). The low p^H did not support proliferation of pathogenic and spoilage microorganism in the juice. The moisture levels for the juice increased as the storage periods increased. This was due to degradation of carbohydrate to produce mild acidic condition due to microbial enzymes. The high moisture value for the unripe pawpaw fruit juice was attributed to low total soluble solids (Adepoju *et al.*, 2006). The protein values ranged from 3.72 to 4.07%. The W_2 sample had the least value (3.72%) and W_0 sample had the highest value (4.07%) and W_1 sample had 4.02%. The protein value for the pasteurized juice at 70°C for 4 min and addition of sodium benzoate at varying storage periods were slightly higher relative to the fresh juice. The fat values ranged from 1.01 to 1.64%. The W_0 and W_1 had high and comparable values (1.67 and 1.54%), The W_2 had the least fat (1.01%). The ash values for the three samples (W_0, W_1 and W_2) were 0.40, 0.72 and 0.97%, respectively. The W_2 had the highest value (0.97%) the W_1 had (0.72%).The W_0 had the least ash (0.40%). The three samples had no fibre. The protein, fat and ash values for juice were higher relative to some of the lesser-known tropical fruits such as black plum (Egbekun *et al.*, 1998; Alobo, 2000), *dialium guineense* (Okegbile and Taiwo, 1990) and sour sop (Onimawo, 2002; Obizoba *et al.*, 2004). The total carbohydrate value varied. It ranged from 1.29 to 3.22%. The W_2 had the least value (1.29%) and W_0 had the highest value (3.22%) W_1 had 2.87%. The carbohydrate value for the juice was lower relative to other fruit juice. Pineapple juice had 14% and sour sop had 15%. The absence of fibre in the juice is not a surprise. This is because it was removed during processing. The values for protein, fat and ash were higher relative to some fruit juices. It is also known that fruits are generally poor sources of these nutrients (Obizoba *et al.*, 2004; Akubor, 1996; Okaka, 1997; Ihekoronye and Ngoddy (1985).

Table 2 presents the effect of sodium benzoate and storage times on micronutrients composition of mature unripe pawpaw fruit juice. The vitamin C increased. The variation ranged from 36.66 to 38.59 mg. The W_2 sample had the least value (36.66 mg) and the W_0 sample had the highest (38.59 mg) followed by W_1 sample which had 38.05 mg. The decrease in vitamin C was from 0.54 and 1.93 mg for W_0-W_2. The vitamin C content of the W_1-W_2 juices did not vary significantly (p>0.05; 38.59 and 38.05 mg). The values were higher (36.66-38.59 mg/100 ml) relative to 18, 24, 25 and 26 mg/100 ml each recorded for banana (Mepha and Akpanumam, 1990), pineapple (Onimawo, 2002; Obizoba *et al.*, 2004) mango (Badifu, 2000; Badifu *et al.*, 2000) and sour sop (Onimawo, 2002).

Table 1: Effect of preservative and storage on proximate composition of mature unripe pawpaw (*Carica papaya*) fruit juice (wet weight)

Nutrients (%)	W_0	W_1	W_2
Moisture	90.64±0.01	90.85±1.00	93.01±0.02
Protein	4.07±0.01	4.02±0.01	3.72±0.01
Fat	1.67±0.01	1.54±0.01	1.01±0.00
Ash	0.40±0.01	0.72±0.01	0.97±0.01
Crude fibre	0.00±0.00	0.00±0.00	0.00±0.00
Total carbohydrate	3.22±0.01	2.87±0.01	1.29±0.01

Means ± SD of 3 determinations.
W_0: Day 1, W_1: Week 1, W_2: Week 2

Table 2: Effect of preservative and storage periods on the micronutrient composition of mature unripe pawpaw (*Carica papaya*) fruit juice

Nutrients mg/100 ml	W_0	W_1	W_2
Vitamin C	38.59±0.76	38.05±0.06*	36.66±0.07*
Thiamin	0.00±0.00	0.00±0.00	0.00±0.00
Iron	4.88±0.00	3.57±0.01*	2.03±0.02*
Iodine	0.00±000	0.00±0.00	0.00±0.00*
Zinc	9.30±0.11	6.10±0.01*	1.95±0.01*
Sodium	38.97±0.52	30.46±0.01*	22.13±0.02*
Potassium	42.09±0.00	28.03±0.02*	16.15±0.01*

Means ± SD of 3 determinations.
Mean values with * in the same row are different ($p<0.05$) from W_0, W_0: Day 1, W_1: Week 1, W_2: Week 2

Table 3: Effect of preservative and storage time on the phytochemical composition (%) of mature unripe pawpaw (*Carica papaya*) treated fruit juice

Phytochemicals	W_0	W_1	W_2
Terpenoids	4.22±0.03	4.16±0.01*	4.35±0.01*
Saponins	3.13±0.02	2.44±0.04*	0.89±0.04*
Flavonoids	5.07±0.02	4.66±0.04*	3.37±0.01*
Tannins	1.47±0.02	0.32±0.01*	3.55±0.02*

Means ± SD of 3 determinations.
Mean values with * in the same row are different ($p<0.05$) from W_0, W_0: Day 1, W_1: Week 1, W_2: Week 2

Table 4: Organoleptic attributes of mature unripe pawpaw (*Carica papaya*) treated fruit juice

Parameter	W_0	W_1	W_2
Colour	7.37±0.23[a]	7.20±0.21[a]	7.19±0.13[a]
Flavour	6.70±0.20[c]	5.03±0.19[b]	4.03±0.22[a]
Taste	6.23±0.26[c]	4.53±0.23[b]	2.47±0.18[a]
Mouthfeel	6.23±0.27[c]	5.81±0.24[b]	3.45±0.21[a]
General acceptability	7.10±0.18[c]	5.10±0.15[b]	2.87±0.21[a]

Means ± SD of 3 determinations.
[abcd]Mean values with different superscript letters in the same rows are different ($p<0.05$). W_0: Day 1, W_1: Week 1, W_2: Week 2

The decreases in vitamin C were not a surprise. It is known that oxidation occurs in foods containing ascorbate. Increase in storage periods decreased the vitamin C in the juice. Vitamin C retention is often used as an estimate for the overall nutrient retention in a food product. This is because it is by far the least stable nutrient. It is highly sensitive to oxidation and leaches into water soluble media during storage (Davey *et al.*, 2000). Ascorbate degrades immediately after harvest and degrades steadily during prolong storage (Murica *et al.*, 2000) even in frozen products (Rickman *et al.*, 2007). The Vitamin C plays important role in general body metabolism, synthesis of haemoglobin, wound healing, intracellular cement substance and stress (Shubhangini, 2008). There was no thiamin in the juice stored at various periods. The vitamin C content of the juice could enhance absorption of iron. Ascorbate reduces and chelates nutrients during food digestion (Hurrel and Egile, 2007). Ascorbate in foods destroys iron inhibitors to increase absorption of iron two to three-fold (Sirgenberg *et al.*, 1991; Stekel *et al.*, 1986). It also enhances iron absorption by reducing ferric III to ferrous (Fe^{2+}), a form of iron readily absorbed. An intake of 100 ml of the juice daily is sufficient to provide 15-60 mg vitamin C as recommended for humans (Fox and Cameroon, 1980). However recommended daily allowance for ascorbate varies with age, sex and physiological status (Fox and Cameroon, 1980).

The juice contained high iron, zinc, sodium and potassium. Storage periods caused significant reduction in these minerals. Iron values ranged from 2.03-4.88 mg. The variation was influenced by time and preservation. The addition of sodium benzoate caused a progressive decrease in iron values weekly. The W_0 had the highest value (4.88 mg) and the W_1 sample had 3.57 mg and the W_2 had 2.03 mg. The differences in ascorbate values of the W_0 and the W_2 samples were relative to each other when compared and was 2.08 mg (4.88 to 2.03 mg). The iron values for the juice for W_1 and the W_2 samples differed ($p<0.05$) from that of the W_0 juice sample. The iron values for the W_0 and the W_2 samples decreased progressively. This could be that phytate or tannins chelated iron in juice. Iron exists mainly as haemoglobin of the red blood cells. It plays an important role in many parts of the body including immune function, cognitive development, temperature regulation and work performance (Groff *et al.*, 1995). The addition of benzoate and storage had varied effects on zinc value for the juice. Benzoate decreased zinc from 9.30-6.10 mg in the W_0 and the W_1 samples. The W_2 sample had the least value (1.95 mg). The difference in zinc value for the W_0 and the W_2 was 7.15 mg. The W_1 and the W_2 values for zinc varied. The slight decrease in zinc regardless of increased storage showed that long storage has little or no adverse effect on zinc. The W_2 juice that had the highest decrease (1.95 mg/100 mg) relative to the W_0 sample might be attributed to increased microflora population usage for metabolism as well as high tannins level in the W_2 sample (3.55%). Sodium values for the W_1 and the W_2 juice samples varied significantly. The range was from 22.13-38.97 mg. W_2 had (22.13 mg) relative to the W_1 sample (30.46 mg) and W_0 (38.97 mg). The decrease was (8.25 mg). The high sodium content of the juice under varying storage periods was associated with the addition of sodium benzoate as a preservative. Sodium benzoate prevents sodium loss in foods. Potassium values differed. The range was from 16.15 to 42.09 mg. The W_2 juice had much more decreases (16.15 mg) relative to W_1 (28.03 mg). The decrease was 11.88 mg when the samples were compared (28.03 is 16.15 mg) (11.88 mg). The potassium values for W_1 and W_2 samples were significantly ($p<0.05$) different from the sample W_0 sounds. The high potassium in the juice was due to sodium benzoate that reduced the rate of loss during storage periods. Regardless of the preservative, potassium was lost due to low carbohydrate value for the juices. It is a commonly observed phenomenon that potassium deficiency is associated low carbohydrate diet (Shubhangini, 2008). Consumption of potassium in diet increases protein retention and protects against hypertension in patients sensitive to high sodium intake (Anderson Young, 2008).

The effect of sodium benzoate and storage time on phytochemical value for mature unripe pawpaw juice (*Carica papaya*) is presented in Table 3. There were both significant reduction and increase in phytochemical values for juice as the storage periods increased. The terpenoids values were a function of time and added preservative. Treatments had varied effects on the terpenoids value for the juice. The addition of sodium benzoate to W_1 sample decreased its terpenoids (4.22-4.16%) as the storage increased. On the other hand, as the storage time increased the W_2 sample value increased from 4.22 to 4.35%. The differences were 0.13 and 0.19% (4.35-4.22 and 4.16%, each). The terpenoids values for juices, W_1 to W_2 were slightly significantly ($p<0.05$) different. Saponins values differed. The range was from 0.89 to 3.13%. Saponins are known to reduce risk for cancer (Vegetarian Nutrition, 1998). Most terpenoids and polyphenol in *Carica papaya* were reported for their antimicrobial properties (Odebiyi and Sofowora, 1979). This property for terpenoids protected the phytochemical value for the entire juice samples regardless of fermentation and microflora spoilage at the various storage periods. Sodium benzonte decreased saponins differently. The W_2 sample had much more decrease (0.89%) reductive to that of W_1 sample (2.44%). The decrease was 2.24% when the samples were compared (3.13-0.89%). The saponins values for the juices, W_1 to W_2 were significantly ($p<0.05$) lower relative to the W_0. The decreases in saponins during zero periods to W_1 and W_2 were attributed to storage and addition of sodium benzoate. It is an established fact that saponins inhibit the Na^{2+} efflux by the blockage of the Na^+ out of the cell. This causes higher concentrations in cells, activating Na^+-Ca^{2+} antiporter to produce elevated cystosolic Ca^{2+} which strengthens the concentration of the heart muscle and reduces congestive heart failure (Schneider and Wolfling, 2004). Flavonoids value was a function of both storage time and preservation. The addition of sodium benzoate caused a progressive decrease in flavonoids value for the juice on weekly basis. The zero time (W_0) sample had the highest value (5.07%) relative to those of W_1 and W_2 values 4.66 and 3.37%. The differences in flavonoids between W_0 and W_2 samples were 0.41% (5.07-4.66%). The W_1 and W_2 flavonoids values for these samples varied relative to the W_0 sample, respectively. The decreases in flavonoids values for preserved juice as the weeks of storage increased (from 5.07-3.37%) for W_0 and W_2 were due to fermentation and production of other compounds related to flavonoids. Flavonoids were reported to have anti-viral, anti-allergic, anti-inflammatory, anti-tumor and anti-oxidant activities (Donald, 2000). The treatments had varied effects on tannins content of the juice as the week increased. Tannins decreased from 1.47-0.32% for W_0 and W_1 samples, respectively. On the other hand, the tannins value for W_2 sample increased its value to 3.55%. The differences were 2.08 and 3.23% (3.55-1.47 and 0.32%, each). The tannins content of the juice from W_1-W_2 varied significantly that of W_0. The increase in tannins might be associated with microbial fermentation that produced increased levels of tannic acid in the juice.

Table 4 presents organoleptic attributes for juice samples from W_0-W_2. The colour value for the samples ranged from 7.19-7.37. The W_0 sample had the highest colour value (7.37) and the W_2 had the least (7.19). The W_0 had the highest value relative to the W_1 and the W_2, the difference was not significant ($p>0.05$). The W_1 and W_2 had comparable colour (7.20 and 7.19). The flavour score for the W_0 sample was the highest (6.70). The W_2 had the least (4.03). There was significant change ($p>0.05$) in flavour of the samples as the time of exposure increased. The mean taste score decreased significantly as the time of exposure increased. The W_0 had the highest taste score (6.23) and the W_2 had the least (2.47). The decrease was much more for the W_2 (2.47). The mean mouth feel score for the juice was highest for the W_0 (6.23) and decreased progressively to 3.45 for the W_2. The W_1 had a mouth feel score of 5.81. The mouth feels score varied significantly with time. The mean general acceptability score varied from 2.87 to 7.10 with time. The W_0 sample had the highest value (7.10) and W_1 and W_2 had 5.10 and 2.87, respectively. Panel responses showed that the juice differed significantly ($p<0.05$) in colour, flavour, mouth feel, taste and general acceptability. The panelist preferred the W_0 juice to any other. The decrease in colour, flavour, mouth feel taste and general acceptability might be that during storage, fermentation occurred to produce undesirable microflora spoilage that caused deterioration of the sensory attributes.

Table 5 presents the effect of storage and preservation on the microflora content of mature and unripe pawpaw fruit juice exposed for various weeks. The increased

Table 5: Effect of preservative and storage time on the microflora content of mature unripe pawpaw fruit juice stored for various weeks

Week	Total viable count (cfu/ml)	Coliform cfu/ml)	Mouldcfu/ml
W_0	0.30 x 10^2±0.33 x 10	0.00±0.00	0.00±0.00
W_1	3.48 x 10^4±0.16 x 103*	0.31 x 10±0.03 x 101*	0.00±0.00
W_2	3.13 x 10^4±0.16 x 103*	0.10 x 10±0.02 x 101*	0.00±0.00

Means ± SD of 3 determinations.
Mean values with * in the same column are different ($p<0.05$) from W_0, W_0: Day 1, W_1: Week 1, W_2: Week 2

microflora value for the juice was directly attributed to storage temperature coupled with the high moisture that formed suitable breeding ground for microbial growth and spoilage. The total viable count of the juice ranged from 0.30 x 10^2-3.13 x 10^4. The W_0 sample had the least value for total viable count. The values for W_1 and W_2 samples for total viable count varied significantly ($p<0.05$) from that of the W_0 sample. The level of coliform was a function of the preservative and storage time. There was no coliform in the W_0 juice sample. The W_1 sample had a sharp increase in coliform levels (0.31 x 10^1 cfu/ml) relative to that of the W_0 sample. The W_2 sample had a sharp decrease in coliform value (0.10 x 10^1 cfu/ml). The coliform values for W_1 to W_2 varied significantly from that of the W_0 sample. There was no mould in the juice. This was due to the effect of preservation and storage time. The W_1 and W_2 samples (0.31 and 0.10 cfu/ml, each) supported coliform growth. This was due to synergistic effects of acidic medium and preservative that militated against its growth. The same explanation might be true for no mould growth in the preserved juices. The levels of TVC (0.30 x 10^2-3.13 x 10^4 cfu/ml), coliform (0.00-0.10 x 10 cfu/ml) and mould (0.00-0.00 cfu/ml) for W_1-W_2 samples were below the maximum levels requirement for NAFDAC and SON, specifications/standards (TVC 3.0 x 105 cfu/ml, coliform 5.0 x 10^2cfu/ml and mould 1 x 10 cfu/ml) for fruit and fruit juices (NAFDAC and SON, 2005).

Conclusion: This study evaluated the effect of preservation and storage time on nutrients, phytochemicals and organoleptic attributes of mature unripe pawpaw fruit juice. Storage at room temperature for 2 weeks precipitated significant reduction in the nutrient, phytochemical and organoleptic attributes of the juice. The keeping quality of the juice with preservative was good within two weeks (W_2). The organoleptic attributes of the juice decreased rapidly during storage and microflora enzymes storage during fermentation. Much more nutrition education is needed to sensitize the public on the nutritional and medicinal properties of mature unripe pawpaw fruit juice. Mature unripe pawpaw juice might be an alternative source for fluid and electrolyte replacement in diarrhea, vomiting and fever. The preservative generally reduced the rate of nutrient loss in the juice. It is recommended that advance level of juice processing should be employed to extend shelf life of the juice and as well as maintain its nutrition profile.

Authors' contributions: This study was carried out in collaboration between all the authors. J.N. Chikwendu and R.O. Edeh designed and supervised the study while O. Ugwuanyi analyzed and did the write up. All authors read and approved the final manuscript.

Competing interests: Authors have declared that no competing interests exist.

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