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Original Article

The effect of garlic and ginger phytogenics on the shelf life and microbial contents of homemade soursop (*Annona muricata* L) fruit juice

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ABSTRACT: The preservative effects of garlic and ginger was compared with that of sodium benzoate in assessing the shelf quality of locally prepared soursop juice. The soursop juice without treatment (T_1) was used as the control while others in four replicates were separately treated with 50 mg/ml garlic (T_2), 50 mg/ml ginger (T_3), mixture garlic and ginger in equal proportion of 50 mg/ml each (T_4) and 10 mg/ml (T_5) sodium benzoate respectively. The microbial counts ranged from 3.0×10^4-1.27×10^6 cfu/ml juices with the untreated recording the highest concentration of contamination compared with the treated juices of which sodium benzoate had the least microbial contamination. The microorganisms consistent in all the treatments were *Bacillus* sp., *Staphylococcus* sp., *Acetobacter* sp., *Klebsiella* sp., *Saccharomyces cerevisiae* and *Candida tropicalis* while the distribution of *Streptococcus* sp., *Klebsiella* and *Penicillium* sp., and *Proteus* sp were sporadic. Marginal decreases in pH values were observed in the stored soursop juices across treatments. The results obtained showed that the treatment of freshly prepared soursop juices with sodium benzoate, and a mixture of garlic and ginger improved storage span and reduced health risks of infection and/or intoxication from their consumption.

KEYWORDS: Shelf life, soursop juice, phytogenics, preservatives, microbial load.

INTRODUCTION

The increase in fruit crop farming and diversity of fruits in many tropical countries coupled with risk of post-harvest losses have given rise to alternative means of processing these fruit into valued products such as beverages, wine, jellies, juice, flakes, jam puree, syrups, ice creams etc. Tropical fruits e.g. soursop, orange, grape, pineapple, cherry, guava, cashew, lemon and watermelon have increasingly gained global importance due to their medicinal, nutrient, flavor, exotic aroma and color (Osemwegie *et al*., 2005, Adeola and Aworh, 2010). In addition to being a dependable nutrient-rich food and accessible as refreshing drink, their trees also provide a comforting shade to people from the burning effect of the sun. They are also explored for income generation by many locals living in developing world in the wood business, retailing of the fruits, other tradomedicinally valuable parts of the tree and fallen branches as firewoods. Fruit crops remain a part of human diet since the beginning of creation and are popular as ornamentals in many African homes (Wenkam, 1990; Okwu and Emenike, 2006).

Soursop is a fruit crop native to the Caribbean and Central America with a biogeographic spread to many tropical areas of the world including the rainforest belt of Nigeria and other parts of Africa. Soursop which is known scientifically as *Annona muricata* L is described by many other common names such as soursap, guanabana, graviola, corossol, guyabano depending on the geographic location of the plant (Samson, 1980). It a heavily fruited, low-branching tree crop belonging to the Family Annonaceae with about 60 representative species and known mostly for its edible fruits

referred to as Anona (Okigbo and Obire, 2009). The fruits are large, variably shaped, highly prized for their pleasant aromatic, juicy flesh, distinctive flavor and may be consumed raw (fresh), cooked or fermented. It could also be processed into different products of economic value among which is juice and it has many therapeutic properties e.g. diuretic, antiurethritis, antihaematuria, antiantibacterial, anticancerous, astringent, sedative, and anti-aging (Asprey and Thornton, 1995; Rapisarda *et al*., 1999). Soursop fruit juice is also reported to be rich in nutrients such as amino acids, vitamins especially ascorbic acid, fibre, proteins, unsaturated fats and essential minerals (Aluko, 1989; Rice *et al*., 1990; Amusa *et al*., 2005). There is however a growing concern about the shelf life and health safety of fruit juices and many fruit products including those from soursop. This concern stems from fruit processing activities involving field and post-harvest handling, especially processing practices such as washing, peeling, shredding and cutting that increase the sources of microorganisms, consequently facilitate deterioration, and compromise their market value (Aluko, 1989; Umme *et al*., 2001; Health Canada, 2006; Chukuezi, 2010). Homemade soursop fruit juice is often extracted as freshly squeezed juice from healthy ripened fruit pulp as an unfermented, untreated and ready to consume product (Melbourne, 2005). Furthermore, asceptic processing of fruit juices led to longer shelf life (Food and Drug Administration, 1999; Ketema *et al*, 2008). The methods of extraction used in the production of soursop fruit juice from the pulp also affect the shelf-life quality (Quek *et al*., 2012). Generally, loss in storage quality is brought about by the activities of microorganisms especially bacteria and fungi (Umme *et al*., 1997; Ikegwu and Ekwu, 2009; Amusa *et al*., 2005). Many factors including the heavily seeded nature of the fruits, fragile and mushy flesh which make them vulnerable to pre- and post-harvest processes were noted in literature to militate against the use of soursop fruits in fruit juice production on both small and commercial scale (Abbo *et al*., 2006). Several techniques popular among which is heat (pasteurization, boiling), exposure to UV radiation, storage under deoxygenated and high pressure state, chilled storage (refrigeration), the use of polyethylene terephthalate (PET), other chemical and synthetic preservatives have reportedly improved the shelf life of processed fruits all over the world (Umme *et al.,* 2001; Medeni, 2006; Quek *et al.,* 2012). Water (hot or cold) and heat (boiling, pasteurization or sterilization) extractions of fruit juice though optimize their shelf life quality but controversially cause loss of their organoleptic property e.g. flavor, viscosity, taste, nutrient etc. Preservatives of chemical origins e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene, tert-butyl hydroquinone, ethoxyquin etc., are popular bacteriostatic and fungistatic in food and drink productions and they have revolutionized the food and eating pattern of humans. Despite these benefits, they have also been linked to food toxicity/poisoning, immune-depression and cancer in humans. This has led to the ban or restriction of permissible uses of artificial (synthetic or chemical) preservatives (nitrates, nitrites, sulfites). These coupled with the possibility of health risk including allergies such as irritation of the skin and eyes, dermatitis, eczema, headaches, hyperactivity etc. from the consumption of juices preserved with chemical or synthetic agents underscore the importance and therefore the drive to seek safer biological means of preserving fruits and fruit products.

The paradigm shift from the ancestral ways of preserving foods by salting, sun and air drying, fermentation, and dependent on natural rather than processed foods involving artificial preservatives in food industries was attributed to civilization, modern hi-tech, expanding demands and food security by Bateman *et al*. (2004). A de-evolution of this trend back to cheaper use of natural biological agents as preservative/additive in fruit, food and animal feed productions was driven by the heretic belief of lack of negative health or environmental consequences of their constant usage (Seetaramaiah *et al*., 2011). This consequently underscores the relevance of biotechnology and exploration of natural preservatives as potentially safe alternatives to chemical products popularly used in food processing.

Literature abounds on fruit crop processing, fruit juice extraction, efficacy of different artificial preservatives (over six thousand) in improving the appearance and storage quality, retarding microbial growth in foods and drinks (Nwachukwu *et al*., 2007; Ogiehor *et al*., 2008; Onyeagba *et al*., 2004). Studies showed that the practice of mixing different exotic fruits and/or certain food preservatives positively impact on the flavor and taste of fruit and fruit products (Nwachukwu *et al*., 2007; Ogiehor *et al*., 2008). Little is however reported in literature on the use of natural products as preservatives in subsistent food and fruit processing especially in fruit juice extraction. This study was therefore carried out to compare the performance of a synthetic (Sodium benzoate) and selected natural (garlic and ginger) preservatives on the shelf life quality of homemade soursop fruit juices.

MATERIALS AND METHODS

Collection of Soursop fruits

Fresh, unblemished and matured soursop fruits were purchased from a metropolitan market in Benin City, Edo State, Nigeria. The soursop fruits were transported in clean paper bags to the laboratory for juice extraction.

Extraction of Fruit Juice

The fruits were washed carefully under flowing tap water, peeled, cut into halves, deseeded and then juiced with a mechanical juice extractor. The expressed undiluted juice was divided into batches; three per treatment, including the control in hitherto dry heat sterilized (160 $^{\circ}$C for 1 hour) jam bottles with airtight stoppers respectively. The treatments include T_1- untreated (control); T_2- garlic (50 mg/ml); T_3-

ginger (50 mg/ml), T_4- a mixture of ginger (50 mg/ml) and garlic (50 mg/ml) and T_5- 0.1% sodium benzoate. The natural additives were previously washed under flowing tap water, air-dried in large square trays for 3 days, slowly oven dried at 60 °C for 6 hours and pulverized into powder with a mechanical grinding machine. The differently treated juices were kept on a laboratory workbench at room temperature (30 ± 1°C) mimicking retail and local consumer home condition for 30 days (4 weeks) and analyzed for pH and the presence of microorganisms per week.

Proximate and pH Analysis

The proximate analysis (quantitative) of the fresh fruits was carried out according to the directions enumerated in AOAC (1980) while 10 ml from each of the treated and untreated fruit juice was dispensed by means of a sterile calibrated glass pipette into a sterile 50ml conical flask and its pH determined with the use of pH meter. The pH meter was standardized using a phosphate buffer of pH 7.

Culture Media Preparation

Twenty eight grams (28 g) of synthetic nutrient agar (NA) and 39 g of potato dextrose agar (PDA) powder were weighed separately using sensitive top-loading balance and each dissolved in 1000ml of distilled water in sterilized pyrex conical flasks respectively. These preparations which were carried out in a recirculating laminarflow chamber (PCR-8) were each shaken continuously to ensure complete dissolution and corked with cotton wool-in- aluminium foil paper stopper to avoid contamination. These were then autoclaved at a temperature of 121 °C for 15 minutes and plated.

Shelf Life Evaluation

One milliliter (1ml) was extracted from each treatment by means of sterile pipette into previously sterilized test tubes containing 9 ml each of distilled water and thoroughly mixed by shaking the test tubes. The sample from each treatment was then serially diluted to 10^{-3} dilution and 1 ml of the 10^{-3} diluent transferred into sterile petri dishes using the pour plate method. The plates were carefully swirled, allowed to mix and solidify under a UV light. The cultured NA plates in triplicate per treatment were then incubated at 37 °C for 48 hours while the PDA plates were kept at 28 °C for 72 hours. Axenic bacteria culture were obtained by aseptically streaking representative isolate of different morphotypes from previous cultured plates onto freshly prepared NA medium which were later incubated at 37 °C for 24 hours. The pure cultures were later used for gram stain and biochemical tests of bacterial isolates.

Identification of Microbial Isolates

Materials used for gram stain include crystal violet (primary stain), gram iodide (mordant), 70% alcohol (decolourizer) and safranin (secondary stain) and the technique was according to Brucker (1986). Biochemical tests such as catalase, oxidase, coagulase, indole, urease, citrate utilization and sugar fermentation tests were carried out according to Cheesbrough (2006) for further identification of bacterial isolates. Fungal Isolates were identified using lactophenol stain, microscopy for comparison of diagnostic characters such as spores, hyphae, presence or absence of septa etc., and colored monographs and identification books of microfungi (St-Germain and Summerbell, 1996; Ellis and Ellis, 1997; Barnett and Barry, 1998).

RESULTS

The fresh juice extracted from the soursop fruits showed high concentration of moisture (75.8%), carbohydrate (13.83%), ash content (8.9%), phosphorus (2.06%), soluble sugar (1.25%), and potassium (1.39%). The results showed low concentration values of crude protein (0.26%), Manganese (0.09%) and zinc (0.04%) as presented in Table 1.

Table 1: Nutrient and mineral composition of soursop fruit.

Proximates	(%)	Minerals	(mg / 100g)
Crude protein	0.26	Potassium (K)	1.39
Crude fibre	0.36	Sodium (Na)	0.12
Lipid	0.85	Calcium (Ca)	0.64
Ash	8.90	Magnesium (Mg)	0.45
Moisture content	75.80	Phosphorus (P)	2.06
Carbohydrate	13.83	Iron (Fe)	0.11
Total soluble sugar	1.25	Zinc (Zn)	0.04
		Manganese (Mn)	0.09
		Copper (Cu)	0.14

Table 2: Bacterial and fungal loads of homemade soursop fruit juices pre-treated with natural and synthetic preservatives during the study.

Bacteria load (cfu/ml)

	Shelf-life after extraction (days)				
	0	7	14	21	28
T_1	$^{a}1.4\times10^{5}$	$^{a}3.2\times10^{5}$	$^{a}4.4\times10^{5}$	$^{a}9.6\times10^{5}$	$^{a}7.0\times10^{5}$
T_2	$^{a}1.3\times10^{5}$	$^{b}2.1\times10^{5}$	$^{a}3.1\times10^{5}$	$^{b}4.5\times10^{5}$	$^{b}6.0\times10^{4}$
T_3	$^{a,b}1.1\times10^{5}$	$^{c}1.1\times10^{5}$	$^{b}1.5\times10^{5}$	$^{c}2.2\times10^{5}$	$^{b}5.0\times10^{4}$
T_4	$^{b}8.0\times10^{4}$	$^{b}1.7\times10^{5}$	$^{b}1.3\times10^{5}$	$^{b}4.0\times10^{5}$	$^{b}4.0\times10^{4}$
T_5	$^{c}4.0\times10^{4}$	$^{c}8.0\times10^{4}$	$^{b}1.0\times10^{5}$	$^{c}2.1\times10^{5}$	$^{b}3.0\times10^{4}$

Fungal load (cfu/ml)

	Shelf life after extraction (days)				
	0	7	14	21	28
T_1	$^{a,b}3.0\times10^{4}$	$^{a}4.1\times10^{5}$	$^{a}1.4\times10^{5}$	$^{a}1.3\times10^{5}$	$^{a,b}1.27\times10^{5}$
T_2	$^{a,b}2.0\times10^{4}$	$^{b}2.4\times10^{5}$	$^{a,b}1.2\times10^{5}$	$^{a}1.16\times10^{5}$	$^{a,b}1.10\times10^{5}$
T_3	$^{a}4.0\times10^{4}$	$^{b}2.9\times10^{5}$	$^{a,b}1.15\times10^{5}$	$^{a}1.07\times10^{5}$	$^{a,b}1.03\times10^{5}$
T_4	$^{b}1.0\times10^{4}$	$^{c}1.0\times10^{5}$	$^{b,c}1.05\times10^{5}$	$^{a}1.03\times10^{5}$	$^{b,c}9.60\times10^{5}$
T_5	$^{a,b}3.0\times10^{4}$	$^{c}5.0\times10^{4}$	$^{c}8.4\times10^{5}$	$^{a}8.9\times10^{5}$	$^{c}7.70\times10^{5}$

T_1 = control, T_2= garlic treated juices, T_3= ginger treated juices, T_4= juices treated with a mixture of garlic and ginger, T_5= sodium benzoate treated juices. Means with similar alphabets in the same column are not significant difference (Student Newman-Keuls *test*)

Twenty-eight days after storage at room temperature, soursop fruit juices treated with garlic (50 mg/ml), ginger (50 mg/ml), a mixture of garlic and ginger (50 mg/ml each) and 0.1% sodium benzoate recorded reduced bacterial and fungal loads compared to the week one microbial profile results. There was a marginal increase in the bacterial load after the first week of incubation compared to the third week.

Table 3: Distribution and occurrence of microorganisms in the various treatments of homemade soursop fruit juices during the study.

Shelf life (days)	Treatment of fresh soursop juice				
	T_1	T_2	T_3	T_4	T_5
0	*Bacillus* sp *Candida tropicalis* *Klebsiella* sp *Pecilium* sp *Staphylococcus* sp	*Bacillus* sp *Candida tropicalis* *Staphylococcus* sp *Penicilium* sp	*Bacillus* sp *Candida tropicalis* *Klebsiella* sp *Pecilium* sp *Staphylococcus* sp	*Bacillus* sp *Candida tropicalis* *Staphylococcus* sp *Penicilium sp*	*Bacillus* sp *Candida tropicalis* *Penicilium* sp
7	*Aspergillus niger* *Bacillus* sp *Candida tropicalis* *Klebsiella* sp *Penicilium* sp *S. cerevisiae* *Staphylococcus* sp *Streptococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *Penicilium* sp *Rhizopus stonolifer* *Staphylococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae* *Staphylococcus* sp	*Acetobacter aceti* *Aspergillus flavus* *Bacillus* sp *Candida tropicalis* *S. cerevisiae* *Staphylococcus* sp	*Bacillus* sp *S. cerevisiae* *Staphylococcus* sp
14	*Acetobacter aceti* *Aspergillus niger* *Bacillus* sp *Candida tropicalis* *Penicilium* sp *Proteus* sp *Rhizopus stonolifer* *Staphylococcus sp*	*Acetobacter aceti* *Aspergillus niger* *Bacillus* sp *Candida tropicalis* *Penicilium sp* *S. cerevisiae* *Staphylococcus* sp	*Bacillus* sp *Candida tropicalis* *Klebsiella* sp *S. cerevisiae* *Staphylococcus* sp	*Acetobacter aceti* *Aspergillus niger* *Candida tropicalis* *S. cerevisiae* *Staphylococcus* sp	*Acetobacter aceti* *Bacillus* sp *S. cerevisiae*
21	*Acetobacter aceti* *Aspergillus niger* *Bacillus* sp *Candida tropicalis* *Lactobacillus* sp *Penicilium* sp *S. cerevisiae* *Streptococcus* sp	*Acetobacter* sp. *Bacillus sp* *Candida tropicalis* *S. cerevisiae* *Streptococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae* *Streptococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae*	*Acetobacter* sp *Bacillus* sp *Candida tropicalis* *S. cerevisiae*
28	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *Lactobacillus* sp *Proteus* sp *S. cerevisiae*	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae* *Streptococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae* *Streptococcus* sp	*Acetobacter aceti* *Bacillus* sp *Candida tropicalis* *S. cerevisiae*	*Acetobacter aceti* *Bacillus* sp *S. cerevisiae*

This pattern is different from what was observed in the fungal load profile, which dropped slightly on day 21 of storage (Table 2).

Decrease in the level of microbial contamination was observed in treated soursop juices compared to the untreated (control). The juices pretreated with sodium benzoate and a mixture of garlic and ginger recorded the least microbial loads respectively. Fungi representatives were increased in diversity towards the end of the study compared to the bacterial isolates. This is affirmed by Newman-Keuls *test* that showed a significant difference in microbial loads of various treatments with increasing shelf-life.

Table 3 shows the microbial (bacteria and fungi) isolates at different stages of the shelf life of the soursop fruit juices. The results showed that *Staphylococcus* sp., *Bacillus* sp. *Acetobacter aceti*, *Saccharomyces cerevisiae*, *Candida tropicalis* and *Apergillus niger* were more consistent throughout the period of study. *Streptococcus*, *Klebsiella* and *Penicillium* species isolates were sporadic in appearance during the study. Fungi were observed to be more dominant (obtrusive) in occurrence than bacteria species from study. The fresh pretreated and untreated juice extracts on the first day of the experiment recorded the presence of *Bacillus* sp., *Staphylococus* sp., *Penicillium* sp. and *Candida tropicalis* respectively.

Table 4: pH values of soursop juices of varying treatment and storage interval of soursop juices.

Treatment	pH values at different shelf life (days)				
	0	**7**	**14**	**21**	**28**
T_1	4.2	4.0	4.3	4.0	5.2
T_2	4.4	4.3	4.4	3.9	3.6
T_3	4.4	4.3	4.4	3.9	3.9
T_4	4.6	4.4	4.5	3.8	3.7
T_5	4.3	4.2	4.6	3.9	3.3

T_1 = control, T_2= garlic treated juices, T_3= ginger treated juices, T_4= juices treated with a mixture of garlic and ginger, T_5= sodium benzoate treated juices.

Table 4 represents the pH value of soursop (*Annona muricata*) fruit juices at different shelf life and this showed a progressive reduction in pH values across treatments and shelf life. The soursop juices treated with synthetic 0.1% sodium benzoate recorded the least pH value (3.3) at the end of the study period.

DISCUSSION

Fruits are consumed for their rich nutritive value. The growing global markets for non-alcoholic fruit juices and transformation of many exotic fruits into juices and/or other easy to consume, preserve and marketable products underscore their economic values. Fruit juices are popular in many tropical countries as thirst quelling, refreshing drinks; nourishing dietary supplements rich in minerals, vitamins, proteins as well as energy rich carbohydrate derivatives. They are widely accepted for their natural sophisticated flavor, food and diverse health benefits (Al-Hindi *et al*., 2011). The report from the proximate analysis of soursop fruits compare favorable with other tropical fruits e.g. pineapples, oranges, grapes, apples, guava, watermelon, cashew banana etc. Bates *et al*. (2001) reported that soursop plants are less globally dispersed contrary to report from Okigbo and Obire (2009) on their status as common ornamentals and fruit trees in many parts of Nigeria, especially the rainforest belt. This proves that they may also compare on the basis of availability, distribution, consumer preference (quality), consumption convenience and yield (Amusa *et al*., 2005; Abbo *et al*., 2006). The nutritive content of soursop fruit juices showed the presence of crude fibre (0.36%), protein (0.26%), carbohydrate (13.83%), ash (8.9%), lipid (0.85%). moisture content (75.80%) and total soluble sugar of 1.25% per 100g of fruits. The range of trace minerals coupled with the nutrient profile identified in the locally extracted soursop fruit juices underscore their acceptability and importance as vital in human diet and health (Asprey and Thornton, 1995; Onibon *et al*., 2007).

The technologies for the commercial production of these juices have been revolutionized with the introduction of ultra-high pressured packaging, refrigeration, chemical preservatives, sterilization (UV-light, pasteurization and boiling) and carbonation (Quek *et al*., 2012). In addition, many of these technologies have been reported to have negative health consequences such as allergies, cancer, food poisoning and organ malfunctions in humans (FDA, 1999). Home-made non-commercial juices that are processed by using mechanical or electrical juice extractor, squeezing, macerating or aqueous/solvent extractions, with or without the application of heat have short shelf life (Abbo *et al*., 2006). This is in congruence with the observed rate of microbial colonization, increasing microbial load records and the shelf life profile registered in the present study. It further supports why studies are expanding on the use of natural preservative in fruit juice preservation. Although, microorganisms associated with locally made and commercially packaged fruit juices have been studied but the inconsistent reports from previous works on the consortium of microorganisms that are responsible for spoilage underpins the role of both human and environmental factors in food processing (Omemu *et al*., 2005; Okigbo and Obire, 2009; Odu and Adeniji, 2013). *Acetobacter aceti*, *Bacillus* sp., *Klebsiella* sp., *Proteus* sp., *Staphyllococcus* sp and *Streptococcus* as well as fungal moulds which were recorded from this study and differ slightly from the microbial profile reports in previous related works. It however supports the observation of quantitative and diverse growth of microbial contaminants with increasing storage period even at room temperature (Abbo et al., 2006; Okigbo and Obire, 2009; Odu and Adeniji, 2013). Although the series of identification methods used for the bacterial isolates which precluded molecular method and the difficulties associated with the preservation of the cultures undermined identification to species level, the knowledge of the species could offer a more interesting interpretation of their negative health consequences.

The differing results on the species of microorganisms associated with biodeterioration of fruit juices and foods from related works may be attributed to a combination of factors

that include the extraction technique, handling (especially during processing), packaging (filling stage) and treatment (heating, acidification, chilling, aeration and/or nature of preservative used). Intrinsic properties of the processed fruits such as the level of initial microbial flora, water activity, oxido-reduction potential, antimicrobial constituent and physical integrity coupled with prevailing environmental conditions could have also contributed to the variations in the composition of microorganisms associated with the shelf life of homemade fruit juices. The near anaerobic condition of the stored soursop juices due to the use of an air-tight stopper could cause selective microecological evolution of microbes isolated from the study. This could be the reason why fermentors such as *Bacillus*, *Streptococcus*, *Candida tropicalis* and *Saccharomyces cerevisiae* were consistent isolates (Jay, 2005). A better knowledge of the biochemical and physiological mechanisms that leads to spoilage of fruits and fruit derivatives such as soursop juice is therefore fundamental to understanding more on the ecological succession dynamics of the biodeteriorants. Wareing and Davenport (2005) in a separate study observed that common isolates of fruit juices migrated from sources related to poor hygiene, pre and post harvest wounds on processed fruits, and the local environment. It is therefore logical to infer that the consistency of *Acetobacter aceti*, *Bacillus* sp., *Candida tropicalis*, *Saccharromyces cerevisiae* and *Staphyllococcus* sp in even fruit juices treated with either chemical (sodium benzoate) or natural (pytogenic) preservatives e.g. garlic, ginger or their mixture could be due to their acquired tolerance capacity and/or the pH of the juice broths. These set of microorganisms were also reported in a related work involving fermentation of soursop fruits (Sunday *et al*. 2010). Consequently, microbial load presence in the soursop juices after the 28^{th} day incubation period increases the chances of food poisoning due to toxin production. It may also facilitate the biodeterioration rate, causing accumulation of metabolic products and moulding. These undermine both the market value and consumer acceptance of the soursop juices (Gow-Chin and Asin-Yan, 1996).

The microbial load data obtained from fruit juices suggest that the natural preservatives (ginger, garlic and their mixture) were less effective in prolonging the shelf life compared to synthetic sodium benzoate preservative. The reason for this observation is not well understood but may be attributed to the insolubility of natural preservatives used for the study. This could synergize the nutrient status of the fruit juices creating the appropriate food base for the colonization by microorganisms (Andargie *et al*., 2008; Fasolin and Cunha, 2012). Adepoju and Karim (2004) showed from their work on *Spondias mombin* (hog plum) that high moisture and carbohydrate content potentially attract many microorganisms as observed from the study. Sodium benzoate, a common chemical preservative showed a better bacteriostatic and fungistatic performance due to the acidic condition of the fruit juices. This is supported by the pH data enumerated in Table 4. Although, Seetaramaiah *et al*. (2011) inventory on natural preservative used in food processing excluded potent medicinal garlic, ginger or their mixture, it is observed from this study that they reduced microbial loads over storage duration when compared to the control. This may be due to the phytochemical property of these plants especially their fruits (Tagoe *et al*., 2010). While this lends credence to previous reports on the medicinal value of these plants or their parts as antimicrobial, more study is required on the mechanism of antibiosis (Onyeagba *et* al., 2004). The fruit preservation performance of these phytogenics showed no significant difference in the reduction of the microbial loads in fruit juices treated with ginger, garlic and mixture of both respectively. It is therefore pertinent to investigate further the concentration at which the each phytogenic is most effective in improving the shelf life and sustaining the organoleptic quality of fruits and fruit products (Brull and Coote, 1999).

The pH results ranged between 3.3 and 5.2 (Table 4) and showed a gradual acidic inclination that was inversely proportional to the microbial load profile in the various fruit juices (Fleet, 2003). Environmental factors coupled with the activities of microorganisms pertinent among which is the production of enzymes, ripening, flavor, pH, ionic strength, moisture content and other organoleptic characteristics of fruits. In addition, the incomplete dissolution of the natural preservatives may also alter, if not significantly, the pH of the juices during storage affect quality (Nunes *et al*., 1995; Okwo *et al*., 2010). The near anaerobic condition of the study may have favored the early appearance of bacteria species and the late emergence of fungi isolates as equally observed in the microbiological results from the work of Essien *et al*. (2011) on a local commercial cereal-based non-alcoholic drink known to Nigerians as *Kunun*. This result supports pH as an important microbial growth factor and determinant of the composition of species isolated per time (Ukwo, 2010).

More information is yet required on the use of pulverized plant parts i.e. seeds, bark, leaves and fruits or their extracts as preservatives of fruit products at ordinary or room temperature. Information on these plant parts as preservatives is presently scarce and their use is unpopular in food industries. This may be connected with their insolubility, nature of the phytochemicals, parts of the plant and methodology (condensation, decoction or extraction) used. Technological information on hot water extraction of fruit juices may lead to improvement in their shelf life but logically affects negatively their nourishment and organoleptic values. Suffice to say, supplements such as sugar, sweeteners (synthetic or natural) and flavoring may be required to restore acceptable sensory qualities of fruit products as currently practiced in most commercial fruit drinks production (Bates *et al*., 2001; Lanciotti *et al*., 2004). In addition, the consortium of microorganisms reported in this study are contaminants associated with poor hygiene practices from both handling (harvesting, transportation and cultural practice i.e. manure, irrigation etc) of fruits coupled with the choice of extraction process. Microbiological quality, safety, and shelf-life of fruit juices destined for consumer markets are subject to approved minimum quality control standards and checks by regulatory agencies in Nigeria such as Nigerian Agency for Food and Drug Administration and Control (NAFDAC). The microbial load data obtained from this study raised some health concerns as they overshot the minimum limit (10^3 cfu/g) reported by Odu and Adeniji (2013) for fruit drinks. This compounded the risks of infection and allergy due to intoxication from their consumption. Most organisms may grow and develop in the fruits used for the juice extraction (normal or emigrant flora) while others use

the fruits either as a secondary host or vehicle for pathogenic infection providing the basis for a more robust microbial diversity. This underpins the relevance of further study on the microbial ecology of fruit juices destined for the consumer market or in storage, risk analyses of contaminated juices, the epidemiology of contaminants and critical microbial concentration range for toxin-infection in humans. Although the negative consequences of microbial invasion of fruit juices far outweigh their benefits, some of the microorganisms recorded in this study could be positively harnessed. Microbial strains such as *Aspergillus, Acetobacter, aceti, Candida tropicalis, Penicillium, Saccharomyces cerevisiae* reported in this study have the potential to improve the quality of fermented and soured foods, leavening food such as bread and can be use as probiotic or bio-additives in animal feeds (Wenkam, 1990, Bates *et* al., 2001, Sunday *et al*., 2010).

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