The Effect of Different Preservative Techniques on the Shelf Life of Prepared Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) Fruit Jam

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ABSTRACT`

Fruits of a tropical herbaceous plant, Citrullus lanatus (Thunb.) Matsum and Nakai, grown in Nigeria were aseptically processed into fruit jam and preserved using Salting, Pasteurization and Heat sterilization. A control was produced which was untreated. The product was stored on the shelf at room temperature 28±4°C and studied at ten days intervals for 60 days. Microfloral profile showed Aspergillus, Penicillum, Mucor, Saccharomyces, Bacillus, Micrococcus and Lactobacillus species across treatments. The microfloral profile reduced with respective preservative treatments, increasing as shelf life increased with heat sterilization being the most effective recording the lowest microbial load of 0 x 10² cfu/g at 28±4°C after 60 days of storage (shelflife). The study showed marginal pH increase as shelf life increased. Results showed that heat sterilization best increased shelf life. The microbial evaluation of the product produced results which suggest consumer acceptability and good market quality similar to other fruit selling product.

KEYWORDS: Fruit jam, microflora, preservative, shelf life, Watermelon,

Introduction

Africa accommodates a rich diversity of tropical and subtropical fruits. The inhabitants have been using the plants resources in various ways. The fruits are eaten raw or processed into fruit products (Pandey, 2004). 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 fruits into valued products such as beverages, wine, jellies, juice, flakes, jam puree, syrups, ice creams etc (Vwioko *et al*., 2013). Tropical fruits such as 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). Technical knowledge has been employed to yield value added products from these plant resources. Such products include fruit juices, syrups, jellies, jams and drinks. One of the very good resources for the production of fruit product is the watermelon fruit.

Citrullus lanatus (Thunb.) Matsum and Nakai var Lanatus commonly called watermelon is an annual herbaceous plant of the cucurbitaceae family widely distributed in the tropical and subtropical regions of the world. It is indigenous to Africa and has been cultivated for over 4000 years (Schippers, 2000). It is a warm seasoned trailing vine with large green and large shaped succulent fruits which are economically the most important part. The edible part is the endocarp which is sweet, juicy and red in colour. This portion constitutes 60% of the fruit and contains water, protein, fat, carbohydrate, minerals, vitamins, fiber and ash (Schippers, 2000). It is used as raw materials for other fruit products because of its high pectin content. In Namibia the fruits are used to satisfy thirst during periods of droughts. In Russia a fermented beverage is made of the juice. In America the fruits are iced as dessert. The pulp is mostly eaten raw by both humans and animals. It is reported to increase the plasma concentration of lycopene and β carotene. It is also applied in local health treatment of malaria and dressing wounds. The seeds are milled and cooked as soup and in making cosmetics (Schippers, 2000). The leaves and rind are cooked as vegetables in parts of Asia. Improvements of yield of watermelon have resulted in a seedless, deep red, crisp flesh with high sugar content and most importantly resistant to fusarium wilt and anthranose diseases. The most popular cultivar sugar baby is resistant to drought. This has resulted to abundance of this fruit more than is being consumed. We may justifiably brag about the high production of this fruit, the loss due to wastage, inadequate utilization and spoilage is high.

There have been reports on the exploitation of watermelon fruits. Literature has dealt on the nutriceutics of *C. lanatus* while its products are not commonly encountered. Reports are common on agro forestry systems and its initiatives at expanding products of fruit crops, promoting food security and alleviating poverty through good business resources, providing employment opportunities and rehabilitating the environment in developing countries (Leakey and Simons, 1998). Technical knowledge has been employed to yield value added product from underutilized plant resources (Pandey, 2004). Fruit syrup extracted from *Chrysophyllum albidum* G. Don (Osemwingie *et al*., 2005), fruit juice production from sour sop (Vwioko *et al*., 2013), manufacture of jelly from fruits, Passion fruit juices and concentrates production amongst others have been carried out. Fruit jam is a gelled product made from juice containing a blended pulp of the fruit. It is one of the oldest and important operations in the fruit product industries. Watermelon is a good raw material for producing fruit jam because of its high pectin content. It affords a means of utilizing large amounts of fruit unsuited for other purposes and also ensuring all year round availability of the fruit with minimal cost.

A major concern for fruit products is spoilage prevention. When spoilage occurs it is usually due to microbial invaders that tolerate fairly dry and sugary environments (Vangrade and Woodburn, 2006). Moulds, yeast and bacteria have been reported to associate with fruit jam spoilage. Raw pulp naturally contains these organisms which are resident on the surface of the fruits. They could be controlled with the use of heat, low temperature and reduction in available water. Their growth is of importance as it may lead to low shelf life and hence consumer unacceptability. The production of jam from fruits is a method of preservation as with bottling and cooling. The acidity and high sugar content makes it an unfavourable medium for the growth of bacteria. Filling jars to the brim with the products so that air is expressed out may prevent spoilage by film forming yeast and acetic acid bacteria. (Anon, 2004). Lactic acid bacteria and moulds can still cause spoilage even after bottling.

Watermelon jam production has not been carried out to any appreciable commercial extent especially in Africa. This may be due to consumer acceptability of end product or spoilage due to low shelf life (Osewengie *et al.* 2005). Hence, this study seeks hygienic processing of watermelon fruit into jam while establishing a condition for good shelf life. Microflora associated and pH of the product is studied at ten days intervals as shelf life increased from 0 to 60 days.

Materials and methods

Collection of samples.

Healthy watermelon and lemon fruits were bought from fruit shops in Benin City, Edo state, Nigeria. They were taken to the laboratory in clean paper bags.

Preparation of fruit jam.

The fruits were washed carefully under flowing tap water. Peeling and juice extraction of the watermelon fruits were carried out. 20kg of watermelon pulp was heated in a sterile kettle to about 80°C for 1hr after which it was allowed to cool to 45°C and filtered using clean cheesecloth to separate the juice. The pulp was then blended. The juice was mixed with 1/2kg of lemon juice pulp and 10kg of sugar. The mixture was shaken vigorously to ensure complete dissolution. Half of the mixture was added to the blended pulp and cooked for 5 minutes. The other half was finally added and cooked until it gelled. Eighty four oven sterilized bottles were each filled with 70g of hot gelled product of which 21 bottles contained the product mixed with sodium chloride (1% w/w), 21 bottles were pasteurized at 60°C for thirty minutes, 21 bottles heat sterilized at 121°C for 15 minutes and the remaining 21 bottles were without further treatments and served as control. The bottled products were stored at room temperature (28±4°C) for 60 days.

Proximate analysis

The proximate analysis (quantitative) of the fresh fruits was carried out. Moisture, protein, fat, fibre, ash, and carbohydrate contents were determined by the methods of AOAC (2005).

Nutritional analysis

Calcium, Phosphorus, iron, potassium, sodium, dietary fibre, vitamin A and C were determined by the methods of AOAC (2005).

pH determination

10g from each of the treated and untreated fruit jam was dispensed into a sterile conical flask containing 100ml of water. 20 ml from each jam mixture was dispensed by means of a sterile calibrated glass pipette into a 50ml beaker 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

62g of sabourand dextrose agar (SDA), 28g of nutrient agar (NA) and 57g of Maconkey agar (MA) were weighed separately using sensitive top-loading balance and each dissolved in 1000ml of sterile distilled water in sterilized pyrex conical flasks respectively. These preparations which were carried out in a recirculating laminar flow chamber (PCR-8) were each shaken continuously to ensure complete dissolution and corked with cotton wool-in- aluminum foil paper stopper to avoid contamination. These were then autoclaved at a temperature of 121 °C for 15 minutes and
plated.

Shelf Life Microfloral Evaluation

1g of each treated and untreated jam was extracted by means of sterile pipette into previously sterilized test tubes containing 9 ml each of sterile 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} diluents 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 were then incubated at 37 °C for 48 hours while the SDA plates were kept at 28 °C for 72 hours. This was done in triplicate per treatments and a fresh bottled jam was taken at random for each study interval. Bacteria and Fungi cultures were obtained by aseptically streaking representative isolate of different morphotypes from previous cultured plates onto freshly prepared MA and SDA media respectively which were later incubated. The pure cultures were later used for gram stain and biochemical tests of bacterial isolates and identification and characterization of fungal isolates. .

Estimation and Identification of Microbial Isolates

Microbial population were enumerated following pour plate method and estimated as number of colonies per gramme of jam using the appropriate dilution factor. 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 (Ellis and Ellis, 1997; Barnett and Barry, 1998). 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 Cheesbrough (2006). 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.

Results

The fresh pulp and juice extracted from the watermelon constitute 60% of the whole fruit. It showed a high moisture content of 93.0% as presented in Table 1. The protein content was observed to be 0.5%, vitamin C 8mg/100g and potassium as high as 100mg/100g.

Table 1: Proximate and Nutritional composition of watermelon pulp.

Proximates	**%**	**Nutrients/ Minerals**		**Minerals**	**mg/100g**
Water	93.0	Dietary fiber	0.4g	Calcium	7
Protein	0.5	Vitamin A	7mg/100g	Phosphorus	10
Fat	0.1	Vitamin C	8 mg/100g	Iron	0.5
Carbohydrate	5.7			Sodium	1
Fiber	0.2			Potassium	100
Ash	0’5				

Table 2 shows the microbial population increasing as shelf life increased. The heat sterilized fruit jam recorded the lowest population.

Table 2: Bacterial and Fungal counts (cfu/g) x10³ of watermelon fruit jam

		Shelf life (days)						
Treatments	**Microbes counted**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
Untreated	Bacteria	0.0	0.0	0.0	2.0	6.3	7.2	7.0
	Fungi	0.0	0.0	0.0	0.0	2.0	3.3	4.5
Salted	Bacteria	0.0	0.0	0.0	1.0	3.0	3.0	3.4
	Fungi	0.0	0.0	0.0	0.0	0.5	3.0	4.1
Pasteurized	Bacteria	0.0	0.0	0.0	0.0	3.2	5.4	5.0
	Fungi	0.0	0.0	0.0	0.0	0.0	2.0	1.0
Heat sterilized	Bacteria	0.0	0.0	0.0	0.0	0.0	0.2	1.0
	Fungi	0.0	0.0	0.0	0.0	0.0	0.0	0.0

In table 3 is presented the distribution and occurrence of microorganisms isolated in the treatments of the watermelon fruit jam. Microfloral assay recorded the presence of the following organisms with *Mucor mucedo* and L*actobacillus sp.* being the predominant fungi and bacteria respectively.

Table 3: Distribution and occurrence of microorganisms isolated in the treatments of watermelon fruit jam.

Shelf life (days)							
Treatments	**0**	**10**	**20**	**30**	**40**	**50**	**60**
Untreated	-	-	-	B1, B2.	B1, B2, F1, F2.	B1, B2, F1, F2.	B1, B2, *B3* F1, F2, F3, F4.
Salted	-	-	-	B1	B1, B2, F1.	B1, B2, F1, F2.	B1, B2, F1, F2, F3, F4.
Pasteurized	-	-	-	-	B1, B2.	B1, B2, F1.	B1, B2, F1.
Heat sterilized	-	-	-	-	-	B2	B2

- = no growth
B1 = *Lactobacillus sp.*
B2 = *Bacillus sp.*
B3 = *Micrococcus sp.*
F1 = *Mucor mucedo*
F2 = *Aspergillus niger*
F3 = *Penicillum sp.*
F4 = *Saccharomyces sp.*

In table 4, the pH of the product increased as shelf life increased. However, pH values higher than the critical safe value of 4.6 for food products were not observed.

Table 4: pH values of watermelon fruit jam of varying treatment and shelf life.

Shelf life (days)							
Treatments	**0**	**10**	**20**	**30**	**40**	**50**	**60**
Untreated	3.76	3.99	4.04	4.09	4.09	4.1	4.28
Salted	3.87	3.96	4.01	4.07	4.06	4.07	4.2
Pasteurized	3.85	3.93	3.99	4.00	4.01	4.01	4.1
Heat sterilized	3.9	3.94	3.99	3.99	3.97	4.0	4.06

Discussion

The proximate analysis of watermelon fruits compared favorably with other tropical fruits like sour sop, pineapples, oranges, grapes and apples. These fruits are consumed for their rich nutritive value. The growing global markets for the transformation of many fruits into easy to consume, preserve and marketable fruit products underscore their economic values. Fruit products 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).

Microfloral assay showed the presence of microorganisms with *Mucor mucedo* and *Lactobacillus sp.* being the predominant fungus and bacterium respectively. The microbial population was observed to increase as shelf life increased. Microflora recorded during this study as shelf life of the fruit product increased compares with what is reported in Osemwingie *et al*., (2005) and Prescott, (2005). Studies have shown that bottled and canned food and fruit products are not 100% sterile (Gaze, 2005). The processes involved is their production are adequate to ensure the destruction of some bacteria but not some mesophiles, thermophiles and spore forming organisms (Schmitt, 1996). These associated microflora are reported in literatures to belong to the groups commonly encountered in processed food and fruit product which although unsterile are considered commercially sterile (Kiiyukia, 2003). Their presence is a reflection that the ambient storage temperature, high moisture content and their affinity for sugar supported their growth. *Mucor sp.* was predominant irrespective of the nature of treatment. This may be attributed to it being a heat resistant spore former. Its presence may establish the presence of other moulds. *Lactobacillus sp.* does not require oxygen for its growth and can still cause spoilage after bottling (singh *et al*., 2004). The pH of the product increased with significant difference between treatments (p=1.69) as shelf life increased. It is typical of some bacillus species to elevate the pH of acid food products (Prescott, 2005). This may have contributed to the growth of some organisms which could be suppressed. However, pH values higher than the critical safe value of 4.6 for food products were not observed.

Physical changes in coloration and aroma were only observed in the untreated and salted jam. In the presence of bacteria the change expected in fruit products at room temperature is alcoholic fermentation by yeast and oxidation of fruit acid by moulds hence change is aroma and colour. Although, salting has been recommended to produce commercially sterile products due to the destructive action of chloride ion, gram negative bacteria are more sensitive. But it reduces the growth of moulds at low pH. Heat treatment is assumed to be adequate to inhibit the organism naturally occurring in fruit products. Hence, pasteurization and heat sterilization of the product was observed to increase shelf life. Increased heat sterilization changes water activity and destroys the enzyme functioning for the occurring microbes. This may have reduced their proliferation.

As it was observed that watermelon fruits can be processed into fruit jam, it was recognized that no single method of preservation gave total sterility. It was possible to reduce microbial load and prolong its shelf life to 60days adopting heat sterilization technique. It has also been demonstrated that the microbial profile of the product under tropical conditions is dominated by spore formers and the product showed visible manifestations of defects. It recognizes that besides acceptable palatability properties which endeared the product to possible consumers' storage shelf life would need to be highly considered before it is good enough for production.

Conclusively, heat sterilized watermelon jam showed less of undesirable qualities indices and therefore higher shelf life may be associated with such. Future investigations to improve the thermally processing and shelf life of this product is required as these fruit can be exploited to yield added products and hence contribute to the economic potentials of fruit of tropical crops.

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