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Microbial Quality Assessment of Fruit Juice Produced from Two Varieties of Watermelon (*Citrullus lanatus*)

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Abstract

Juice was produced from two varieties of watermelon. It was pasteurized, preserved with 0.1% sodium benzoate chemical, refrigerated (4°C), stored for 7 days and was analyzed for its microbiological qualities. Total aerobic bacterial counts ranged from $1.5 \pm 0.1 \log_{10}$ cfu/ml for water melon sugar baby juice (A6P), $2.0 \pm 0.1 \log_{10}$ cfu/ml for water melon orangeglo juice (6AP) and control $1.2 \pm 0.15 \log_{10}$ cfu/ml orange juice (PA6). Coliform counts were $1.0 \pm 0.2 \log_{10}$ cfu/ml A6P, $1.5 \pm 0.05 \log_{10}$ cfu/ml for 6AP, $1.0 \pm 0.03 \log_{10}$ cfu/ml PA6, *Staphylococcus* counts were $1.0 \pm 0.13 \log_{10}$ cfu/ml, $1.4 \pm 0.01 \log_{10}$ cfu/ml and $1.0 \pm 0.06 \log_{10}$ cfu/ml, no counts were recorded for *Salmonella* count, while the yeast and mold counts ranged from $1.0 \pm 0.02 \log_{10}$ cfu/ml A6P to $1.2 \pm 0.03 \log_{10}$ cfu/ml 6AP, $1.0 \pm 0.03 \log_{10}$ cfu/ml PA6. Bacteria isolated were *Bacillus subtilis*, *Lactobacillus plantarium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas fluorescence* and *Micrococcus sp* while the mold isolates were *Rhizopus nigrican* and *Mucor mucedo*. The yeast isolate was *Saccharomyces cerevisiae*. The total aerobic count does not exceed the standard of ICMSF of 10^5 cfu/ml of foods, hence combination of chemical preservation and the use of pasteurization reduces the microbial load to the minimal and thus makes it shelf stable.

1. Introduction

Juices are aqueous liquids expressed or otherwise extracted usually from one or more fruits [1]. Juices are prepared mechanically by squeezing or macerating the pulp of fresh fruits or vegetables without application of heat or solvent to give an unfermented clouded, unclarified and untreated juice ready for consumption. Diluting or blending is a common practice as many fresh juices are either too acidic or too strongly flavored to be pleasant for consumption [2]. Fresh fruit and vegetable juices are an important part of modern day diet in many parts of the world as they are rich source of nutrients such as vitamins, minerals and other naturally occurring phytochemicals which are of health and therapeutic benefits [3]. The consumption of fruit juices could have both positive and negative effects on the part of consumers. Fruit juices processed under hygienic conditions could play important role in enhancing consumer's health through inhibition of breast cancer, Congestive Heart Failure (CHF) and urinary tract infection [1]. However, freshly extracted juices may not always be safe owing to the heavy load of microbes. Major ingredients of juices such as water, sugar, natural fruit pulp, etc may also carry some microbial contaminants which may cause spoilage of drinks or

gastrointestinal disorders to consumers [2].

Watermelon fruit juice is the liquid extract of watermelon fruit which are filtered to remove pulp or fiber; it is a delicacy in some countries like Mexico, some of which even comes as a perfect blend with other additive fruit juice [4]. Watermelon *Citrullus lanatus* (Thumb) also known as Nigger melon is a vine-like (*Scrambler and Trailer*) flowering plant originally from Southern Africa. It belongs to the family of cucurbitacea and the genus *Citrullus* [5]. According to [6], watermelon fruits are classified according to the different varieties which are yellow watermelon, the moon and stars variety, cream of Saskatchewan, melitopolski, Icebox and dansuke. Icebox watermelons are bred to feed one person or a small family, and as such much smaller than their counterparts which are in the range of 5-15 pounds. Watermelon plant varieties in this genre includes the Sugar Baby and the Tiger Baby. Sugar baby watermelons are sweet pulped with dark green rinds and were first introduced in 1956. It is a delicious super sweet, early maturing small fruit, bearing 'Icebox watermelon'. The flesh of this watermelon variety is fine textured and very sweet. The vines are also shorter than big fruited watermelon vines which are ideal for those with limited space. Orangeglo are yellow/orange fleshed watermelon plant varieties, they are typically round and can be both seedless and seeded. Orangeglo variety has a very sweet orange pulp, and is a large oblong fruit weighing 9–14 kg [7]. It has a light green rind with jagged dark green stripes. It takes about 90-100 days from planting to harvest [8]. The common watermelon fruit is round with reddish mesocarp having a lot of seed and is mostly common in the south. There are various species with different coloured endocarp for example, red flesh, yellow flesh and orange flesh. It contains vitamin B1 and B6, potassium and magnesium in addition to vitamin A and C which is generally common to all fruits and vegetables [9]. Watermelon (*Citrullus lanatus*) is rich in carotenoids. Carotenoids have antioxidant activity and free scavenging property thereby help in reducing the risk of cancers, cardiovascular diseases, arteriosclerosis diabetes and arthritis and protects against macular degeneration.

A watermelon is nominally 60% flesh and about 90% of the flesh is juicy which contains 7 to 10% (w/v) sugar. Thus, over 50% of the watermelon is readily fermentable liquid [10]. Watermelon has lot of water which makes it an ideal medium for contamination by microbes and it can get contaminated during cutting and packaging with harmful bacteria and water used in fields. *Salmonella*, *Listeria*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Shigella*, *Fusarium Oxysporum*, *Candida*, *Staphylococcus* etc can be seen in water melon, some causing spoilage and others causes contamination leading to one disease or the other. Microbial spoilage may be due to plant pathogens cutting on the fruit or other parts. There are no serious bacteria or viruses disease of watermelon. Fungus diseases are the major diseases that are associated with the fruit and some of them are; Fusarium wilt caused by *Fusarium* (a common cause of

poor harvesting) which can be controlled by rotational cropping, Anthracnose which is a destructive disease of watermelon, and other cucurbits. The different types of spoilage which are common in fruit drinks are ripeness, rot, discoloration, sliminess, putrefaction, whiskery and fermentative spoilage [11]. The common method for preserving the juice to prevent spoilage or microbial attack are; canning, pasteurization, concentrating, freezing, evaporation, spray drying and the use of chemical preservatives [12].

Microbial and other form of spoilage may be delayed or prevented by various methods of preservation, such methods help to retain the nutritive value of the product, extend shelf-life and keep it safe for consumption [13]. Watermelon is a fruit with high moisture content [14] and this characteristic makes it highly susceptible to microbial spoilage caused by gram positive bacteria which are very sensitive to low acidity. The alarming rate of food poisoning, food contamination, effect of watermelon on health, its varieties, the microorganisms and the nutrients present in it are unnoticed at all by people. In light of these, this research was carried out to establish the differences that exist between watermelon fruit juices which are beneficial to health and how to prevent contamination through principles of good hygiene and other preservative methods. The work is also aimed at producing watermelon juice hygienically with the hope of maintaining the microbial quality and shelf stable.

2. Materials and Methods

2.1. Collection and Processing of Fruits

Mature, ripe healthy water melon and orange fruits were bought from different sales point in Sayedero Markets, Ilaro, Ogun State, Nigeria. Weighing of watermelon fruits was done separately using a manual weighing balance. The sugar baby and orangeglo watermelon weighs 2.5kg and 4kg respectively. The watermelon and orange fruits were washed with distilled water to remove adhering soils, dirt and extraneous materials and then washed with 5% hypochloride solution and immediately rinsed again with distilled water. The fruits were peeled and cut into small pieces and the seeds were removed.

2.2. Production of Watermelon Fruit Juice

The fruits were cut vertically into slices with a sterile stainless steel knife. The green outer rinds (epicarp) were removed from the mesocarp (peeling) with the aid of a knife. The peeled rinds were weighed to know the weight of the fruit. Weight of sugar baby and orangeglo varieties of watermelon are 0.3kg and 0.5kg respectively. The fleshy part of the fruit's (mesocarp) edible portion was removed, cut into small pieces, transferred into a sterilized blender (Moulinex model) and blended until sufficient juice was produced. The entire slurry was transferred into a sterile muslin cloth to sieve off the particles. The clear liquid obtained was

transferred into clean sterile air tight bottles.

2.2.1. Treatment of Fruit Juice

Chemical preservative (sodium benzoate) 0.1% was incorporated into the fresh watermelon juice filled into bottles and capped immediately. Pasteurization was done at 80°C for 15 minutes to destroy microorganisms and was cooled and stored in the refrigerator at 4°C.

2.2.2. Production of Orange Juice

The oranges were weighed (weight obtained was 2.5kg (2,500g), washed, peeled with sterile stainless steel knife, cut and squeezed using a squeezer. Muslin cloth was used to sieve the pulped juice to obtain a clear juice and chemical preservative was incorporated. The juice was pasteurized at 80°C for 15 minutes and bottling of the juice was done. It was cooled and stored for 7 days.

2.3. Microbial Analysis

2.3.1. Total Aerobic Bacteria Count

The microbiological analysis was carried out on the Day 0 fresh samples and Day 7 of the stored samples according to [15]. Nutrient agar was used for enumeration of bacteria. Ten fold dilution of each watermelon juices were made using 0.1% peptone water. 1ml aliquot from appropriate dilution was transferred aseptically into sterile petri dishes. To each plate about 15ml of melted and cooled Nutrient Agar was added. The inocula was evenly mixed with media by rotating the plates and allowed to solidify. The inverted plate was incubated for 48 hours at 37°C. The Total bacteria count (cfu/ml) was determined using a colony counter.

2.3.2. Total Coliform Bacteria

MacConkey agar was used for the detection of coliform bacteria. 1 ml from each appropriate dilution was transferred aseptically into sterile petri dishes. To each plate, 15mls of the molten medium was added swirl and allow to solidify. The inverted plate was incubated for 48hrs at 37°C.

2.3.3. Total *Samonella* Count

Bismuth sulphite agar was used for the detection of *Samonella*. 1ml aliquots from each appropriate dilution was transferred aseptically into sterile petri dishes. To each plate, 15mls of the molten medium was added swirl and allow to solidify. The inverted plate was incubated for 48hrs at 35°C.

2.3.4. Total *Staphylococcus* Count

Baird-parker agar was used for the enumeration of *Staphylococcus*. 1ml aliquots from each appropriate dilution was transferred aseptically into sterile petri dishes. To each plate, 15mls of the molten medium was added swirl and allow to solidify. The inverted plate was incubated for 48hrs at 35°C.

2.3.5. Yeast and Mold Enumeration

Potato dextrose agar (PDA) was used for enumeration of yeast and mold. 1ml aliquots from each appropriate dilution

was transferred aseptically into solidified PDA plates. Samples were spread all over the surface of the plates using sterile bent glass rod. The plates were then incubated for 48 to 72hrs at 28°C. Counting (cfu/ml) was carried out by using colony counter [15].

2.3.6. Statistical Analysis

Data were reported as Mean±SD. Statistical significance was established using One-Way Analysis of Variance (ANOVA) at 5% level of probability and differences between means were compared using Duncan Multiple range test.

3. Results

The Microbial analysis of the watermelon fruit juices made from sugar baby and orangeglo varieties of watermelon and the single strength orange juice used as control were presented in Table 1. There was no growth of microbes at Day 0 of fresh produced samples while at the Day 7 growth was observed. The total aerobic bacteria counts for sample A6P Sugar baby, 6AP Orangeglo, and PA6 Orange juice are $1.5 \pm 0.1 \log_{10}$ cfu/ml, $2.0 \pm 0.1 \log_{10}$ cfu/ml and $1.2 \pm 0.15 \log_{10}$ cfu/ml respectively. Coliform count range from $1.0 \pm 0.2 \log_{10}$ cfu/ml, $1.5 \pm 0.05 \log_{10}$ cfu/ml, $1.0 \pm 0.03 \log_{10}$ cfu/ml. *Staphylococcus* count range from $1.0 \pm 0.13 \log_{10}$ cfu/ml, $1.4 \pm 0.01 \log_{10}$ cfu/ml, $1.0 \pm 0.06 \log_{10}$ cfu/ml with no growth of *Samonella* and the yeast and mold count (fungi count) are: $1.0 \pm 0.02 \log_{10}$ cfu/ml for A6P, $1.2 \pm 0.03 \log_{10}$ cfu/ml for 6AP and $1.0 \pm 0.03 \log_{10}$ cfu/ml for PA6. From the analysis, the Anova $P < 0.05$ shows that there was significant difference in the mean count among the microorganisms isolated from the two varieties of watermelon.

Table 2 shows the biochemical characterization of bacteria isolates associated with stored refrigerated watermelon juice at the 7th day. This result shows that most of the isolates were positive to the biochemical test. All isolates were positive to catalase test and most of the bacteria isolates were gram negative rods, except *Bacillus sp* and few gram positive cocci. Microorganisms isolated were *Bacillus subtilis*, *Lactobacillus plantarium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas fluorescence* and *Micrococcus sp.*, while the mold isolates were *Rhizopus nigrican* and *Mucor mucedo*. The yeast isolate was *Saccharomyces cerevisiae*. Figure 1 shows the percentage occurrence of microorganism in stored refrigerated watermelon juice from two varieties of watermelon at the 7th day. Among the bacteria, *Bacillus subtilis*, had the highest occurrence of 12%, and *Klebsiella pneumonia* 12%, *Lactobacillus plantarium* 10%, *Pseudomonas fluorescence* 10%, *Proteus vulgaris* 10%, *Staphylococcus aureus* 8%, and *Micrococcus sp* 8%, while the mold isolates were *Rhizopus nigrican* 6% and *Mucor mucedo* 4%. The yeast isolate had the highest occurrence of *Saccharomyces cerevisiae* 20%.

Table 1. Microbial analysis of stored fruit juice produced from two varieties of watermelon.

(log ₁₀ cfu/ml)					
L Samples	Total Aerobic Bacteria	Coliform count	Salmonella count	Staphylococcus count	Yeast and mold count
A6P	1.5 ± 0.1 ^b	1.0 ± 0.2 ^c	NG	1.0 ± 0.13 ^b	1.0 ± 0.02 ^c
6AP	2.0 ± 0.1 ^a	1.5 ± 0.05 ^a	NG	1.4 ± 0.01 ^a	1.2 ± 0.03 ^a
PA6	1.2 ± 0.15 ^c	1.0 ± 0.03 ^b	NG	1.0 ± 0.06 ^c	1.0 ± 0.03 ^b

Key: A6P - Fruit juice produced from Sugar baby variety of water melon

6AP – Fruit juice produced from Orangeglo variety of watermelon

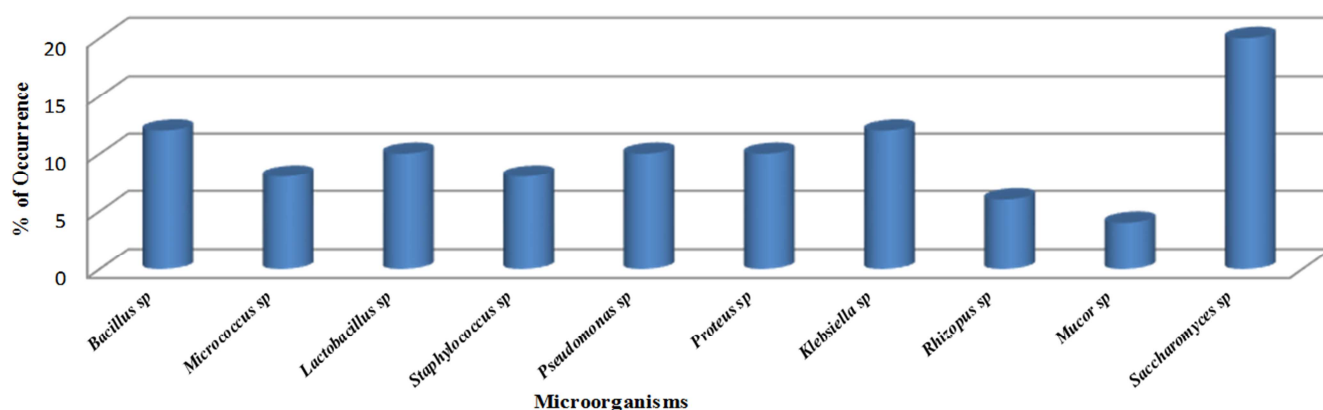
PA6 – Fruit juice produced from Orange juice

Day 0; No growth of microbes observed

Day 7; Microbial Analysis presented above

Table 2. Cultural and biochemical characteristics of bacteria isolated from stored watermelon juice.

Suspected Microbes	Shape	Gram reaction	Catalase	Oxidase	Coagulase	Motility	Urease	Indole	Spore	Suc	Glu	Gal
<i>Bacillus subtilis</i>	Rod	+	+	+	-	+	+	-	+	+	+	+
<i>Micrococcus sp</i>	Cocci	+	+	+	-	-	+	-	-	+	+	-
<i>Staphylococcus aureus</i>	Cocci	+	+	-	+	-	+	+	+	+	+	+
<i>Lactobacillus plantarium</i>	Rod	+	+	-	+	+	+	+	-	+	+	+
<i>Pseudomonas flourescence</i>	Rod	-	+	-	+	+	+	-	-	+	+	+
<i>Proteus vulgaris</i>	Rod	-	+	-	+	+	+	+	+	+	+	+
<i>Klebsiella pneumonia</i>	Rod	-	+	-	-	+	+	-	-	+	+	+

**Figure 1.** Percentage occurrence of microorganisms in stored refrigerated water melon juice.

4. Discussion

The Microbial analysis of the watermelon fruit juices made from sugar baby and orangeglo varieties of watermelon and the single strength orange juice used as control were presented in Table 1. The total aerobic bacteria counts for sample A6P Sugar baby, 6AP Orange glo, and PA6 Orange juice shows significance difference at $P < 0.05$. It could be deduced that sample 6AP Orangeglo has the highest microbial count due to high water content, sugar content and high pH followed by sample A6P and lastly sample PA6 which has the lowest count. This can be due to the presence of citric acid in the fruit which acts as preservative. The *Coliform*, *Staphylococcus* and the fungi count from the two varieties of watermelon shows significant difference at $P < 0.05$ and the orangeglo had the highest count, while there was no growth of *Salmonella* in all the samples. This makes this juice safer. The mean count of the yeast and mold of the two varieties also shows significant difference at $P < 0.05$ while Orangeglo

watermelon had the highest mold and yeast count Table 2 shows the microbes isolated from the samples which are in the genera: *Bacillus*, *Lactobacillus*, *Klebsiella*, *Micrococcus*, *Pseudomonas*, *Staphylococcus*, *Rhizopus*, *Mucor*, *Saccharomyces*. This agrees with the report of [16]. The presence of these microorganisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates, such as sugar which often leads to the production of acid after fermentation [17], they are normal contaminants of water melon fruit starting from the farm to the processing plant. According to International Commission for Microbiological specification for Food (ICMSF), microbial standard count should not be more than 10^5 cfu/ml for total plate count and this implies that all the analyzed samples conforms to this standard. The total aerobic counts of all juice samples were lower than the standard due to preservation and refrigerated made juice. The same applies to proliferation of coliforms in the freshly made juice. Microorganisms isolated and percentage of occurrence from stored watermelon juice are presented in

Figure 1. Among the bacteria, *Bacillus* and *Klebsiella* species were more frequently encountered. This agrees with the report of [18] that *Bacillus* is a major spoilage organism in juices. The presence of *Staphylococcus aureus* in the juice could be attributed to its wide spread in the environment. It could also be as a result of contamination from handlers. *Staphylococcus aureus*, a mesophile has been implicated in food poisoning outbreak of some food materials [19]. The occurrence of *Klebsiella sp* could be due to contamination from equipment since it is a common equipment contaminant. *Pseudomonas sp* are commonly found on the fruit surfaces which can end up in the juice during production. They are able to grow on a wide variety of organic substrates and are regular components of food spoilage [20]. *Micrococcus sp* occurs in a wide range of environment including water, dust and soil, this could be due to contamination from water during washing of the fruits.

The molds and yeasts isolated were *Mucor mucedo*, *Rhizopus nigrican* and *Saccharomyces cerevisiae* implicated fungi as contaminants of fresh fruits especially in the presence of injuries. The microorganisms had been earlier reported to be isolated from water melon juice [21]. In addition, the ability of these organisms to survive in acidic juices at both ambient and refrigerated temperature (8°C) and low pH value has been documented [21]. Water and environment may play a major role in fungi contamination of watermelon especially during washing of the fruits [22]. The presence of *Saccharomyces cerevisiae* is expected due to its preference for sugar. Besides, lower pH highly favors yeast proliferation [20].

5. Conclusion

The freshly made watermelon juice from the two varieties reveal that the orangeglo samples contained high amount of microbes than the sugar baby watermelon and orange juice. The production of juice from water melon is important in that it will reduce wastage of the fruits by farmers and provide nutrient to their respective consumers. It also reduces economic loss in the country. The results suggest that watermelon juice pasteurized and treated with sodium benzoate reduce the microbial count and makes it safer while sugar baby varieties recorded lower count, therefore, its production should be encouraged.

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