Microbiological And Nutritional Evaluation Of Water Melon Juice (Citrullus lanatus)

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Abstract: Studies were conduct on different samples of water melon juice (fresh water melon juice with sugar (FWMJS), fresh water melon juice without sugar (FWMJWS), refrigerated water melon juice with sugar (RWMJS) and refrigerated water melon juice without sugar (RWMJWS). The result of the investigation obtained showed that the highest bacteria load of 5.3X 10^6 cfu/ml, fungal count of 1.2X10^6 cfu/ml and yeast count of 1.0X10^6 cfu/ml were recorded in RWMJUS after 15 days of storage. The identification process revealed that microorganisms such as *Eschericca coli* (20%) *Staphylococcus epidermidis* (16%) *Saccharomyces cerevisiae* (12%) and *Aspergillus niger* (4%) were implicated. The nutritional analysis showed that FWMJWS have the highest % crude protein (0.71), ash (0.10), and moisture content (69.75). However the RWMJS contained the highest quantities of Ca (0.51mg/100g), K (1.31mg/100ml), Na (0.26mg/ml), P (0.156mg/ 100ml) Fe (0.30mg/100ml) Zn (0.018mg/100ml) and Mg (0.012mg/100ml) similarly the quantities of Ca (0.51 mg/100g) and Mg (0.012 mg/100g) in FWMJS were the same with that of RWMJS. Study therefore concluded that fresh water melon juice was more susceptible to microbial attack compared to water melon juice fortified with sugar.
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1. **Introduction**

Water melons are consumed fresh, leading to the rejection of water melons that have any visible defect (Williams 2007). All foods, even when properly packaged, undergo biochemical, physical, and other changes that can affect their quality and safety (Sam Saguy and Micha Peleg , 2009). Low temperature slows down the growth of microorganisms and the rate of chemical changes in food which can cause food spoilage (Anon, 2005).

Water melon (Citrullus lanatus) was described by Roger (2006) as *Citrullus lanatus* and is reported to contribute to the proper functioning of the kidney. Pulp chemical composition has been reported as having 91.5% moisture, 0.2% protein, 0.1% fat (reported as ether extracts) and 0.25% total ash (Uddin and Nanjundaswamy, 1982). Another study (Taper et al., 1985) found that watermelon pulp yield was 57.2% and it was composed of 91.2% moisture, 0.69% protein, 0.48% fat, and 0.3% ash.

The different types of spoilage which are common in fruit drinks are ropiness, rot, discoloration, sliminess, putrefaction, whiskery and fermentative spoilage (Parry and Pasway, 1984).

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 (Singleton, 1997). Water melon is a fruit with high moisture content (Erukainure et al., 2010) and this characteristic makes it highly susceptible to microbial spoilage caused by gram positive bacteria which are very sensitive to low acidity (Mossel et al., 1995).

Mossels et al. (1988) reported that during cold storage, the shelf life of the product is determined by temperature, for example, the lag time for growth of *Listeria monocytogenes* at 10^0C is 15 days while at 1^0C the lag time is 3 days. It was further reported that at 10^0C the generation time of the microorganism is 5-8h, while the generation time is between 62 and 131h at 1^0C. Similarly a pH increase from 4.5 to 6.5 decreases the lag time from 60h to 5h.

However this research work is intended to provide information on the microbiological and nutritional evaluation of the different treated products of water melon juice.

2. Material and Methods

Procurement of Sample

Healthy ripe water melon fruit was purchased from Bodija market in Ibadan, Nigeria and transported to the laboratory for subsequent study.

Method of Extraction of Juice from water melon fruit

The fruit was washed with distilled water after which it was washed with 5% hypochloride solution and immediately rinsed again with distilled water. The whole fruit was cut longitudinally using a sterile knife and 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 muslim cloth to sieve off the particles. The clear liquid obtained was transferred into clean sterile airtight bottles.

Treatment of Fruit Juice

Five hundred milliliters of the fruit juice was dispensed separately into four sterile Five hundred milliliters Erlenmeyer flask which were airtight-sealed. Twenty grams of sucrose was added to the fruit juice in two of the airtight Five hundred milliliters Erlenmeyer flask while the fruit juices in the others were without sucrose. The four Erlenmeyer flasks were labeled accordingly and two of them were transferred into a refrigerator at $4^0c \pm 2^0c$ for 15 days while the remaining two flasks were not refrigerated.

Isolation and identification of isolates

Isolation of microorganisms from the water melon juice samples was carried out by sampling at 2 days intervals for 15 days. The media used for isolation included MacConkey agar, Blood agar, Salmonella-Shigella agar, Mannitol salt agar, Yeast extract agar and Potato dextrose agar. The methods of Meynell and Meynell (1970) and Harrigan and Macane (1976) were employed for the serial dilution and pouring of plates respectively. Pure cultures of the isolates were obtained by repeated sub- culturing. The bacterial isolates were identified based on morphological and biochemical characteristics and with reference to Bergey's manual of systematic bacteriology (Sneath, 1986). The yeast isolates were identified using methods described by Sanni and Lonner (1993). The fungal isolates were identified based on macroscopic and microscopic appearance, and with reference to Funder, (1961) and Barnett and Hunter (1972).

Nutritional Analysis of the Fruit Juice

The crude protein, fat, ash, moisture and mineral composition contents were determined using the methods described by A. O. A. C. (2000).

Sensory evaluation of refrigerated water melon Fruit Juice

Sensory evaluation was conducted on the four water melon juice samples. The sensory evaluation panel used was nine undergraduate students of the Department of Microbiology University of Ibadan Nigeria who were regular consumers of water melon juice. The panelists were directed to rate the samples on the bases of appearance, flavor, aroma and overall acceptability. Nine-point Hedonic scale ranging from like very much (9 points), to dislike very much (1 point) were used as described by Larmond (1977). The results obtained were analyzed based on variance using one way ANOVA. Differences between the means were sorted out using Duncan's multiple range test (Duncan, 1955).

3. Results

Table 1 shows the different microorganisms (bacteria, mould and yeast) present in water melon juice with sugar and without sugar stored at refrigerated temperature (4 ± 2^oC) for 15 days. The result revealed that the highest microbial count was observed at 15 day in both the two samples of water melon juice. The bacteria count in water melon juice without sugar was 5.3×10^6 cfu/ml while 2.6×10^5 cfu/ml was recorded in water melon juice sample with sugar. Also the mould count was higher (1.2×10^6 cfu/ml) in the water melon juice sample without sugar while the mould count of 1.0×10^5 cfu/ml was recorded in the water melon juice sample with sugar. Similarly yeast count was higher (1.0×10^6 cfu/ml) in water melon juice without sugar than in water melon juice with sugar (8.0×10^4 cfu/ml).

Further identification of the microorganisms found in the refrigerated water melon juice samples revealed that *E. coli* had the highest percentage occurrence of 20% followed by *Klebsiella pneumonia and Staphylococcus epididermis* which showed 16% occurrence each and the least (4%) was observed in *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus* and *Rhizopus oligosporus* (Table 2).

The highest percentage crud protein of 0.71 was recorded in fresh water melon sample without sugar while the least (0.67%) was noted in water melon juice sample with sugar. The result of the fat content showed that the highest percentage fat (0.05%) was seen in fresh water melon juice sample with sugar while the least was seen in water melon juice without sugar refrigerated for 15 days. There was no observable change in the content of the crude fiber in all the samples analyzed in this work. However, the highest ash content of 0.10 % was observed in fresh water melon juice sample without sugar and the least was observed in refrigerated water melon juice sample without sugar. The percentage moisture content of 69.75 was noticeable in fresh water melon juice sample without sugar while the least (60.80) was observed in refrigerated water melon juice sample with sugar. Refrigerated water melon juice sample without sugar stored for 15 days recorded the highest percentage Carbohydrate content of 99.24% while the least (98.69%) was observed in fresh water melon juice sample without sugar (unrefrigerated) (Table 3).

From the mineral composition analysis results potassium content was reveled to be the highest with 1.31 mg/100ml in refrigerated water melon juice with sugar while the least was noted in both fresh water melon juice without sugar and refrigerated water melon juice without sugar (1.10 mg/100ml). This was followed by calcium showing a value of 0.51 mg/100ml in both fresh water melon juice sample with sugar and refrigerated water melon juice with sugar while the refrigerated water melon juice without sugar recorded the least calcium content of 0.46mg/100ml. However the comparative result showed that magnesium was the least (0.010mg/100ml) as seen in refrigerated water melon juice without sugar (Table 4).

The sensory evaluation result showed that the fresh watermelon juice with sugar was scored highest in terms of taste (7.8±0.00) and overall acceptability scoring (8.9±0.36) closely followed by fresh water melon juice without sugar which showed the highest score for colour and aroma recording 8.5±0.11 and 9.0±0.18 respectively while the refrigerated water melon juice without sugar stored for 15 days had the least score for colour and aroma (Table 5).

Table 1: Microbial load of refrigerated water melon juice samples

Juice samples		**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 11**	**Day 13**	**Day 15**
Juice without Sugar	Bacterial count (cfu/ml)	1.8×10^{2}	1.4×10^{5}	1.3×10^{6}	2.1×10^{6}	2.9×10^{6}	3.7×10^{6}	4.8×10^{6}	5.3×10^{6}
	Mould count (cfu/ml)	2.0×10^{1}	7×10^{3}	3×10^{4}	4.0×10^{4}	6.0×10^{4}	5×10^{5}	8×10^{5}	1.2×10^{6}
	Yeast count (cfu/ml)	1.0×10^{1}	2.0×10^{3}	2.0×10^{4}	3×10^{4}	5×10^{4}	4×10^{5}	7×10^{5}	1.0×10^{6}
Juice with Sugar	Bacterial count (cfu/ml)	5×10^{1}	6.0×10^{2}	9.5×10^{3}	1.2×10^{4}	4.9×10^{5}	6.3×10^{5}	9.5×10^{5}	2.6×10^{5}
	Mould count (cfu/ml)	No Growth	2.0×10^{1}	7.0×10^{2}	8.0×10^{2}	4.0×10^{4}	6.0×10^{4}	8.0×10^{4}	1.0×10^{5}
	Yeast count cfu/ml)	No Growth	1.0×10^{1}	2.0×10^{2}	4.0×10^{2}	2.0×10^{4}	3.0×10^{4}	5.0×10^{4}	8.0×10^{4}

Table 2: Percentage occurrence of microorganisms in refrigerated water melon juice

Microorganisms		**Number**	**% of Occurrence**
Bacteria	*Klebsiella pneumonia*	4	16%
	Eschericia coli	5	20%
	Proteus vulgaris	3	12%
	Staphylococcus epididermis	4	16%
Fungi	*Aspergillus niger*	1	4%
	Aspergillus flavus	1	4%
	Aspergillus ochraceus	1	4%
	Rhizopus nigrican	2	8%
	Rhizopus oligosporus	1	4%
Yeast	*Saccharomyces cerevisiae*	3	12%
Total			100%

Table 3: proximate composition of different samples of watermelon juice

Parameters Determined	**Fresh without sugar (FWMJWS)**	**Fresh with sugar (FWMJS)**	**With Sugar Refrigerated For 15 days (RWMJS)**	**Without Sugar Refrigerated For 15 days (RWMJWS)**	
% Crude protein	0.71	0.67	0.70	0.68	
% carbohydrate	98.69	98.77	99.19	99.24	
% fat	0.041	0.05	0.03	0.02	
% crude fibre	0.02	0.02	0.02	0.02	
% ash	0.10	0.08	0.06	0.04	
% moisture content	69.75	66.83	60.80	68.75	

Table 4: Mineral composition (mg/100ml) of different samples of watermelon juice

Parameters Determined	Fresh without sugar (FWMJWS)	Fresh with sugar (FWMJS)	With Sugar Refrigerated For 15 days(RWMJS)	Without Sugar Refrigerated For 15 days(RWMJWS)
Ca	0.48	0.51	0.51	0.46
K	1.10	1.25	1.31	1.10
Na	0.021	0.025	0.026	0.020
P	0.147	0.153	0.156	0.147
Fe	0.24	0.27	0.30	0.27
Zn	0.010	0.014	0.018	0.015
Mg	0.011	0.012	0.012	0.010

Table 5: Sensory evaluation result of the different water melon juice samples

Samples	Taste	Colour	Aroma	Overall acceptability
Fresh watermelon juice without sugar(FWMJWS)	5.4±0.06b	8.5±0.11d	9.0±0.00d	7.7±0.22c
Fresh watermelon juice with sugar(FWMJS)	7.8±0.05d	7.5±0.16c	8.7±0.92c	8.9±0.36d
Refrigerated watermelon juice without sugar stored for 15 days(RWMJWS)	6.3±0.04c	7.0±0.18a	6.0±0.88a	7±.0±0.44b
Refrigerated watermelon juice with sugar stored for 15 days(RWMJS)	5.0±0.01a	7.2±0.15b	6.1±0.75b	6.8±0.53a

4. Discussion

From the study conducted on different samples of water melon juice, it was discovered that bacteria such as *Eschericia coli, Proteus vulgaris, Klebsiella sp, Staphylococcus epidermidis* and fungi such as *Aspergillus niger, Aspergillus flavus, A. ochraceus , Penicillum sp, Rhezopus sp, Fusarium spp* and *Sacchromyces cerevisiae* are contaminant implicated in the fresh water melon juice with sugar and without sugar refrigerated at 4 ± 2^0C and stored for 15 days.

E. coli and *Klebsiella* species had been earlier reported to be isolated from water melon juice (Nwachuhwa et al., 2008) while Buchet (1995) reported that *E. coil* are found to contaminate fruit and vegetables. In addition, the ability of these organisms to survive in acidic juices at both ambient and refrigerated temp (4^0C) and low pH value had been documented (Nestes et al., 2001).

The microbial load increased from day 1 to 15^{th} day of storage in both sugar and non sugar fortified water melon juice. It was observed that the increase was higher in the water melon juice without sugar than the sugar fortified water melon juice sample. The occurrence might be due to the fortification of the water melon juice with sugar which serves as a curing agent for the juice (Mikey, 2012).

The dominance of *E. coli* in water melon juice samples observed in this study is an indication that *E. coli* might had invaded the juice from sources such as equipment, container, fruits skin and non-compliance with hygienic rule/aseptic during juice extraction process.

The fresh water melon juice sample recorded the best proximate parameters. The lowest crude protein content observed in the stored water melon juice without sugar might be attributed to the storage effect under cool temperature. The microflora associated with the sample could have degraded the juice protein content under storage condition thereby resulting in low protein content. The observed high carbohydrate level in water melon juice fortified with sugar might be due to the fortification of the juice. The addition of sugar can even inhibit the microbial activities which can responsible for breakdown of carbohydrate for growth. The water melon juice without sugar refrigerated for 15 days reflected the lowest ash content. This occurrence can be adduced to reason such as the fermenting activities of the organisms (Omafuwbe et al., 2004). It could be inferred from this occurrence that a low concentration of phytate and oxalate content occurred with increased elemental minerals since these component are fractional constituents of ash (Bergheofer and Werzer 1986). Eka (1980) and Adebowale (1988) reported that low ash content can aid better growth performance and feed utilization efficiency and might also aid high amount of metabolisable energy, since ash is a measure of total inorganic matter in food.

The decrease in moisture content in water melon juice with sugar refrigerated for 15 days had been earlier documented by Yau et al. (2010). This observation might be caused by the fortification of the juice with sugar inhibiting the growth of microorganisms which can utilized carbohydrate present in the water melon juice for respiration which create an unsuitable environment with low water activity for growth (David and Vermit 1989; Uzochukwu et al., 2000). High water content in foods has however been identified as a potential source of spoilage (Barber et al., 1988; Sanni and Ogbonna 1991).

The high content of calcium and potassium observed in water melon juice samples is important because calcium play significant role in cell regulation, maintainers of cell structure and cell differentiation process (Dominguez, 2004). Watermelon flesh contains 11 minerals and 19 vitamins, while the rind is dominant in vitamin C, providing 2 percent of the recommended daily amount (Lee 2011). Since watermelon has a high water content, its juice is a natural diuretic and may help to cleanse the kidneys and bladder of impurities (Lee 2011).

Francis (1995) stated that color influences other sensory characteristics, which subsequently account for food acceptability, choice, and preference. The sensory evaluation result revealed that the fresh melon juice with sugar recorded the highest scored in teams of taste and overall acceptability. Colour is the most obvious change that occurs in many fruits during storage (Yau et al., 2010). In addition, microorganisms such as bacteria, yeast and mould implicated in spoilage of fruits drink caused reduction in organoleptic and quality of the substance which makes them unacceptable to consumers (Curiale 1998; Jideani and Jideani 2006). Results from this study indicate that fresh water melon juice was more susceptible to microbial attack compared to water melon juice fortified with sugar and decreased in nutritional content was noted in the stored water melon juice.

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