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Microbial Analysis, Mineral Contents, and Sensory Quality of *Citrus sinensis* (ORANGE) and *Cocos nucifera* (COCONUT) Blended Fruit Juices

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Abstract

The study was conducted to prepare mixed fruit juice by using coconut and orange juices. A total of six (6) samples of fruit juices were analysed: Pasteurized coconut and orange juice, unpasteurized coconut and orange juice, pasteurized coconut juice, unpasteurized coconut juice, pasteurized orange juice and unpasteurized orange juice. The mineral content of the fruit juice samples such as Calcium, Magnesium and sodium were also analyzed using spectrophotometric methods. Pour plate method was used to analyze the samples to determine their microbiological safety. Identification of gram negative, oxidase and the conventional biochemical testing for gram positive and gram negative bacteria were carried out. Antibiotic sensitivity test was determined by disc diffusion method. The results revealed that sample A with pasteurized coconut and orange juice secured the highest score on sensory evaluation and showed the best consumers acceptance. Bacteria isolated include *Bacillus megaterium, Bacillus polymx, Flavobacterium aquantile* and *Bacillus cereus* while fungi isolated includes: *Saccharomyces cerevisae* and *Candida valida*. Antibiotic sensitivity test carried out on the isolates revealed that many of the bacteria manifested an intermediate to sensitive reaction to most of the antibiotics tested. All the gram-positive bacteria were sensitive to

Gentamycin, ofloxacin and most were resistant to cefuroxime, ceftazidime and Erythromycin. All the gram-negative isolates were sensitive to Ciprofloxacin and ofloxacin. The mineral content of the fruit juice samples such as Calcium, Magnesium and sodium were also analyzed using spectrophotometric methods. Transmission of resistant bacteria is a potential concern with unhygienic handling of fruit juice. Better handling process and health education is crucial to preventing spread of resistant bacteria and food poisoning through these otherwise commonly consumed fruit juice.

Keywords: Coconut, Orange, Pasteurized, Unpasteurized, Bacteria, Fungi, Antibiotics.

Introduction

Fruits are parts of flowering plants derived from the fertilization of specific tissues such as one or more ovaries (Mauseth *et al*., 2003). Fruits have been a part of human diet and food supplement over the years. They are good sources of essential elements which are very important for our body to make body function properly, such as water, vitamins (A, B1, B2, C, D and E), minerals (Ca, Mg, Zn, Fe, K etc.) and organic compounds (Okwu and Emenike, 2006; Dosumu *et al*., 2009). Fruits are also great sources of antioxidants which are responsible for scavenging free radicals. Due to high perishability, fruits become rotten quickly and preservation is also essential to make them available for a long period of time (Brett *et al*., 1996). They are highly perishable, non-staple foods which make-up about 39% of the food intake of people living in developing countries (Bates and Morris, 2001). Fruits and vegetables are important to good health for all age categories, where they constitute one of the most important portions in a healthy diet (Jan and Masih, 2012). Consuming fresh juices is increasing all over the world due to their freshness, high vitamins content, low caloric consumption and ability to reduce risk of many diseases (Rathnayaka, 2013). Such as heart diseases, cancer and diabetes (Rathnayaka, 2013). Fruit juice is a popular drink as it contains antioxidants, vitamins and minerals that are essential for human beings (Aneja *et al*., 2012). The high potassium and low sodium content of most juices help in maintaining a healthy blood pressure.

Vitamin C is naturally present in juices which are essential for the body to form collagen, cartilage, muscle and blood vessels. It also helps in the absorption of iron (IFFJPU, 2011). Juice formulation is one of the methods that could be used to improve the nutritional quality of the juice, this method can improve the vitamin and mineral contents according to the fruits blended together (De Carvalho *et al*., 2007). Apart from nutritional quality improvement, blended juice can be served as appetizers. Phytochemicals found in vegetables and fruits provide a synergistic and additive effects as a result of their significant antioxidant potential (Liu, 2003). Synergistic and additive effects of phytochemicals in vegetables, fruits, and whole grains are held responsible for its potent anticancer and antioxidant activities. Use of a wide range of vegetables, fruits, and whole grains on a daily basis is considered a practical strategy to optimize ones health (Liu, 2012). Based on fruits antioxidant capacities, they are used as indicators for healthy nourishment as well as protection factors of the human body against oxidative destruction (Costescu and Rivis, 2006).

Materials and Methods

Description of the Study Area

The study area was in Igboora, Oyo State. Igboora is one of the seven towns in Ibarapa landand it is located in a derived savanna zone of 7º 15᾽ N and 3º 30’ East of the equator with an average annual rainfall of 1278mm and annual month temperature of 27ºC (Sanusi, 2011). The major part of the project was carried out in the Research Laboratory of Oyo State College of Agriculture and Technology, Igboora.

Sources of Raw Materials / Sample Collection: Fully matured, fresh coconut and orange fruits was procured from Towobowo market at Igboora, Oyo State, Nigeria. They were transported to Oyo State College of Agriculture and Technology Research Laboratory for subsequent analysis. Chemicals and reagents used for this study were of analytical grades.

Extraction of Coconut Water from Coconut: The coconuts were washed thoroughly with water to remove the sand and dirt on them after which they were washed with chlorine water to reduce the microbes that might be on the coconuts. The coconut water was extracted by cracking the coconut shell and the coconut water obtained was stored in a refrigerator at temperature of 4°C in a sterilized plastic container.

Preparation of Coconut Milk Extracts: The method described by (Belewu and Belewu, 2007) was used in the extraction of coconut milk. Coconut milk was prepared

by shelling the nut and the flesh were separated from the shell using a dull sterilized knife. The brown skin was removed from the coconut flesh with a clean razor blade and the flesh was thoroughly washed and later blended using a sterile blender. The blended flesh was put in a bowl and a little amount of warm water was added and left for a few minutes to extract the oil, milk and the aromatic compounds with sieve cloth. The extract was later filtered with 0.18 mm sieve and squeezed, so as to obtain a milky white opaque emulsion with a sweet coconut flavour while the shafts were discarded.
Preparation of Orange Fruit Juices: The fruits were selected and washed with 5% hypochlorite solution and rinsed thoroughly with distilled water. They were peeled with sterile stainless knife, cut into half and squeezed to extract the liquid from the orange. It was then sieved using a sieve cloth to remove the particles and seeds. The juices were bottled in an airtight screw cap sterilized glass bottles and refrigerated at 5°C prior to analysis and to prevent fermentation.

Preparation of Coconut/ Orange Juices Blends

The coconut milk and water extracts were blended with orange juice in varying proportions such as:
sample A: 50:50 percent of orange extract and coconut extract (Pasteurized)
sample B: 50:50 percent of orange extract and coconut extract (Unpasteurized)
sample C: 100:0 percent of coconut extract without orange extract (Pasteurized)
sample D: 100:0 percent of coconut extract without orange extract (Unpasteurized)
Sample E: 0:100 percent of orange extract without coconut extract (Pasteurized)
Sample F: 0:100 percent of orange extract without coconut extract (Unpasteurized)
The blends was homogenized, bottled and some pasteurized at 80°C for 10 minutes in a thermostatically controlled water bathe, cooled at room temperature (27°C) and finally stored in a refrigerator at 5°C until analyzed following the procedure of Emelike and Ebere (2015b)

Preservation of the Formulated Mixed Fruit Juices: The formulated fruit juice was preserved by pasteurizing the drinks at a temperature of 80°C for 10 minutes. The pasteurized formulations were then filled in glass bottles which had tightly fitted lid and stored in a refrigerator at a temperature of 5°C as described by Emelike and Ebere (2015b).

Shelf life Determination of the Formulated Mixed Fruit Juices: The shelf life of the final product was determined by checking the pH weekly, observation of gas production, change of colour and odour for a period of 1 month. The pH was used because when fermentation starts to take place there is the formation of alcohol which shifts the pH towards the basic region because of the ROH (alcohol) functional group as described by Labuza and Labuza (2004).

Microbial Analysis of the Formulated Mixed Fruit Juice: Microbial analysis was carried out on the fruit juice produced to check for bacterial and fungal counts and whether the pasteurization is adequate. Some of the samples were kept on the shelf (room temperature) and some in a fridge of an average temperature of 8°C. The growths of microorganisms in the samples were determined for a period of 2 weeks.

Isolation, Characterization and Identification of Bacteria: An aliquot extract of the coconut and orange and the mixture of the pineapple and coconut extract were serially diluted using the pour plate method of inoculation; 1 ml of each was diluted into 10^{-5} using sterile distilled water. 10^{-4} dilutions were then pour plated on Nutrient Agar plate and 1 ml of 10^{-3} on Potato Dextrose Agar plate for the respective isolation of bacteria and fungi from each of the fruit juice before and after pasteurization. The bacteria plates were incubated at 37°C for 24 hours and fungi cultivation plates at room temperature (28 ± 2°C) for 72 hours. 0.1 ml of each juice sample were obtained aseptically, diluted and plated. At days 0, 5 and 14, serial dilution was carried out on each bottle of the orange, coconut extracts and the mixture of orange and coconut extract respectively and 0.1 ml of 10^{-3} for fungal growth and 10^{-4} for bacterial growth were plated and incubated at 37°C for 24 hours for bacteria and for fungi at room temperature for 72 hours. The colonies obtained for bacteria were counted using colony counter and each distinct colony from each plate were purified by sub-culturing on nutrient agar plate to get a pure culture and then the pure cultures were then transferred into nutrient agar slant and stored at 4°C for later use. The isolates were identified using morphological and biochemical tests according to Bergeys Manual of Systematic Bacteriology (2004).

Identification of Fungi: Two drops of lactophenol blue with cotton wool was dispensed on a clean grease free slide. Using a sterile inoculating loop, a mycelia mat was transferred on the fluid and pressed so that it mixes well with the strain. The mycelia mat was then covered with a clean cover slip. The slides were observed under low power objective of microscope.

Sensory Analysis of the Formulated Mixed Fruit Juice: The sensory analysis was carried out using 10-member panelists; which were done after pasteurization; it consisted of five males and five females whose ages ranged from 20 to 30 years of regular juice drinker, who were trained for one week with pasteurized orange juice, pasteurized coconut extract, and the final mixed fruit juice samples that were formulated. The panelists consist of students of Oyo State College of Agriculture and Technology, Igboora, Oyo State, Nigeria. The sensory qualities evaluated were: Color, Flavor, Taste and Overall acceptability. The coconut/orange juice blends were served with clean glasses to individual panelist. The order of presentation of samples to the panel was randomized and they were instructed to express their feelings about the samples by scoring the sensory attributes like flavor, aroma, taste, colour, mouthful and overall acceptability, potable water was provided to rinse the mouth between evaluations. Each sensory attribute was on a 9-point Hedonic Scale with (9: like the most, 8: like very much, 7: like moderately, 6: like slightly, 5: Neither like nor dislike, 4: Dislike slightly, 3: Dislike moderately, 2: Dislike, 1: Dislike extremely (Iwe, 2010).

Determination of Minerals: Minerals present in the fruit juice samples such as Calcium (Ca) and Magnessium (Mg) were determined using Atomic Absorption Spectrophotometer (AAS) model (Hitachi 170-10), while Sodium (Na) and Potassium (K) were determined by flame photometer as prescribed by AOAC (2011).

Statistical Analysis

Data collected was analysed using descriptive analysis such as mean and standard deviation.

Results

Result of Analysis of Sensory Profiling

Table 1a: Socio-Demographic Characteristics of Panelists

Characteristics		N
Sex	Female	10
Tribe	Yoruba	8
	Igbo	2
	Total	10

Mean ± SD = 20.1 ± 2.8

Table 1b: Mean Result (Mean ± SD) of Sensory Evaluation

Samples	Appearance	Colour	Flavour	Taste	Overall acceptability
A	7.5 ± 1.281	7.7 ±1.010	7.7 ± 1.100	8.1 ±1.356	8.3 ± 1.616
B	6.6 ± 1.685	6.8 ±1.166	7.2 ± 1.249	8.0 ± 0.632	7.9 ± 1.136
C	7.2 ± 1.360	7.2 ± 1.400	7.4 ± 1.200	8.0 ± 0.700	8.0 ± 0.640
D	6.9 ± 1.446	7.1 ± 1.300	7.3 ± 1.269	7.8 ± 1.077	6.7 ± 1.000
E	7.1 ± 0.943	7.4 ± 1.005	7.1 ± 0.943	8.0 ± 0.831	7.9 ± 0.700
F	6.6 ± 1.428	7.5 ± 1.432	7.3 ± 1.005	7.4 ± 1.010	8.0 ± 0.775

Mean ± Standard Deviation

Keys:

Sample A: 50:50 percent of orange extract and coconut extract (Pasteurized)

Sample B: 50:50 percent of orange extract and coconut extract (Unpasteurized)

Sample C: 100:0 percent of coconut extract without orange extract (Pasteurized)

Sample D: 100:0 percent of coconut extract without orange extract (Unpasteurized)

Sample E: 0:100 percent of orange extract without coconut extract (Pasteurized)

Sample F: 0:100 percent of orange extract without coconut extract (Unpasteurized)

Table 2: pH Values Of Stored Orange/ Coconut Juice.

Samples/pH	Week 1	Week 2	Week 3
A	6.03±0.125	5.63±0.125	5.08±0.150
B	6.50±0.263	5.63±0.125	5.20±0.071
C	4.67±0.125	4.00±0.000	3.85±0.042
D	5.01±0.163	4.33±0.236	4.00±0.047
E	7.70±0.163	6.00±0.000	5.49±0.087
F	7.43±0.262	6.05±0.408	6.00±0.125

Table 3: Result of Microbial Analysis.

Samples	Day One (I)		Day Fourteen (After spoilage)	
	Bacteria	Fungi	Bacteria	Fungi
A	$28x10^{-4}$	$5x10^{-3}$	$40x10^{-4}$	$10x10^{-3}$
B	$56x10^{-4}$	$10x10^{-3}$	$60x10^{-4}$	$15x10^{-3}$
C	$18x10^{-4}$	$4x10^{-3}$	$65x10^{-4}$	$7x10^{-3}$
D	$28x10^{-4}$	$7x10^{-3}$	$78x10^{-4}$	$10x10^{-3}$
E	$20x10^{-4}$	$3x10^{-3}$	$85x10^{-4}$	$6x10^{-3}$
F	$48x10^{-4}$	$11x10^{-3}$	$90x10^{-4}$	$15x10^{-3}$

Table 4: Antibiotic Sensitivity Profile for Gram-positive Isolates.

Isolate/Antibiotics	CAZ	CRX	GEN	CTR	ERY	CXC	OFL
Concentration	30µg	30µg	10µg	30µg	5µg	5µg	5µg
Bacillus megaterium	R	R	19.0	R	R	R	23.5
Bacillus polymx	R	R	R	R	R	R	22.5
Bacillus megaterium	R	R	15.0	R	R	R	22.5
Flavobacterium aquatile	R	R	R	5.0	R	R	20.5
Bacillus cereus	R	R	10.0	R	R	R	R
B. megaterium	R	R	16.0	R	R	R	22.5

Diameter of zone of inhibition was measured in millimeter (mm)

Keys:

CAZ: Ceftazidime, GEN: Gentamycin, CTR: Ceftriaxone, ERY: Erythromycin, OFL: Ofloxacin, AUG: Amoxicillin/ clavunate, AMP: Ampicillin, CPR: Ciprofloxacin, NIT: Nitrofuratoin, CRX: Cefuroxime, R: Resistant.

Table 5: Antibiotic Sensitivity Profile for Gram-negative Isolates.

Isolate/Antibiotics	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
Concentration/Zone of Inhibition	30µg	30µg	10µg	5µg	5µg	30µg	30µg	10µg
Bacillus megaterium	R	R	19.0	22.5	22.5	R	17.0	R
Bacillus polymx	R	R	R	20.5	24.5	R	R	R
Bacillus megaterium	R	R	20.0	25.5	24.5	20.0	R	R
Flavobacterium aquatile	R	R	R	21.5	22.0	R	R	R
Bacillus Cereus	R	R	R	R	R	R	21.5	R
B. megaterium	R	R	16.0	16.5	19.5	R	17.0	R

Diameter of zone of inhibition was measured in millimeter (mm)

Keys:

CAZ: Ceftazidime, GEN: Gentamycin, CTR: Ceftriaxone, ERY: Erythromycin, OFL: Ofloxacin, AUG: Amoxicillin/ clavunate, AMP: Ampicillin, CPR: Ciprofloxacin, NIT: Nitrofuratoin, CRX: Cefuroxime, R: Resistant.

Table 6: Mean ±SD of Mineral Content of Samples

Metal Samples	A	B	C	D	E	F	RDA(mg/day)
Sodium (Na)	1.25±0.009	0.63±0.025	1.32±0.009	0.52±0.009	1.18±0.152	0.81±0.014	1000-1500
Magnessium (Mg)	1.13±0.092	0.13±0.025	0.40±0.000	0.70±0.000	0.19±0.005	0.13±0.139	110-300
Calcium (Ca)	1.62±0.016	0.70±0.000	0.90±0.000	0.45±0.012	0.93±0.025	0.51±0.005	800-1100

Keys: *RDA- Recommended Dietary Allowance

Table 4.1 above showed that 100% of the panelists that carried out the sensory evaluation were female and majority of them were of Yoruba ethnic. Eight (8) panelist were mainly Yoruba and Two (2) were Igbos. The meaning of this distribution is that the fruit juice samples were evaluated largely by Yoruba and mainly by female gender that are noted to be very sensitive with taste of substance. Therefore, it was shown from table 4.1 that the panelists who carried out the sensory evaluation preferred sample A (pasteurized coconut and orange fruit juice sample).

From Table 4.1b, the result showed that, juice of sample A (50:50 percent of orange extract and coconut extract (Pasteurized) was characterized with the highest score in all the parameters: color (7.7±1.010), taste (8.1±1.356), flavor (7.7±1.100), appearance (7.5±1.281) and overall acceptability (8.3±1.616), followed by sample C (100:0 percent coconut extract without orange juice {pasteurized}), E (Pasteurized orange juice) and D (unpasteurized coconut juice). The least accepted one was sample B (unpasteurized coconut and orange juice) (color (7.7±1.010), taste (8.0±0.632), flavor (6.8±1.166), appearance (6.6±1.685) and overall acceptability (7.2±1.36). This might be due to the taste of the coconut and orange fruit juice which seems nourishing and good to taste.

From Table 4.2, the result showed that after three weeks ofstorageatroom temperature the pH of sample A was changed from 6.03 to 5.08, samples B from 6.50 to 5.20, sample C from 4.67 to 3.85, sample D from 5.01 to 4.00, sample E from 7.70 to 5.49 and lastly, sample F from 7.43 to 6.00. The overall range of pH is 3.85 - 7.70 with most figures being between 4.00 and 6.50.Sample E had the highest pH value of 7.70, followed by sample F (7.43)while the lowest pH was observed in sample C (3.85).The acidity might be increased due to degradation of carbohydrates present in mixed fruit juice by the action of microorganisms.

It was shown in Table 4.3 that the highest bacterial count was seen in sample B ($56x10^{-4}$), followed by sample F ($48x10^{-4}$), sample A and D ($28x10^{-4}$), sample E ($20x10^{-4}$) while the lowest value was seen in sample C ($18x10^{-4}$) and fungi count present in sample F ($11x10^{-3}$) was characterized as the highest followed by sample B, D, A, C while the least was seen in sample E ($3x10^{-3}$) for day one analysis, this is due to the pasteurization process undergone by the some of the fruit juice samples. After storage for about fourteen days, sample F has the highest bacterial count ($90x10^{-4}$) while sample B ($60x10^{-4}$) has the lowest. For the fungal count, sample F and B ($15x10^{-3}$) has

the highest and sample E ($6x10^{-3}$) has the lowest count. The increase in the bacterial load of the samples may be caused by prolong storage and increased acidity which aids the growth of microbes.

From table 4.5, the result showed that in sample A (coconut + orange (pasteurized) and sample B (coconut + orange (unpasteurized), the isolates *Bacillus megaterium* and *Bacillus cereus* are both susceptible to Gentamycin with the values 19.0mm and 10.0mm respectively while others are resistant. In sample C (Coconut pasteurized), Gentamycin and Ofloxacin are susceptible while others are resistant. Furthermore, sample D (Coconut unpasteurized) is resistant to all other antibiotics except Ofloxacin with values of 22.5mm. The table also showed that sample E (Orange pasteurized) is susceptible to Gentamycin (15.0mm) and Ofloxacin (22.5mm) but resistant to the rest while sample F (Orange Unpasteurized) is resistant to all antibiotic except Ceftriaxone (5.0mm) and Ofloxacin (20.5mm). Moreover, the antibiotic with the highest zone of Inhibition is Ofloxacin (23.5mm) while the lowest zone of inhibition was seen in Ceftriaxone (5.0 mm).

In sample A (coconut + orange (pasteurized), the isolates *Bacillus megaterium* was susceptible to Gentamycin, Ciprofloxacin, Ofloxacin and Nitrofuratoin with the values 19.0mm, 22.5mm, 22.5mm and 17.0mm respectively, while others are resistant. Sample B (coconut + orange (unpasteurized), the isolate *Bacillus cereus* was susceptible to only Nitrofuratoin (21.5mm)while others are resistant. In sample C (coconut pasteurized), *Bacillus megaterium* was susceptible to Gentamycin (20.0mm), Ciprofloxacin (25.5mm), Ofloxacin (24.5mm) and Augumentin (20.0mm) while others are resistant. Furthermore, sample D (Coconut unpasteurized), the isolate, *Bacillus polymx*, was resistant to all antibiotics except Ciprofloxacin (20.5mm) and Ofloxacin (24.5mm). The table also showed that sample E (Orange pasteurized) was susceptible to Gentamycin, Ciprofloxacinand Ofloxacin but resistant to the rest while sample F (Orange Unpasteurized) was susceptible to Ciprofloxacin and Ofloxacin but resistant to other antibiotics (Table 4.5.1).Moreover, the antibiotic with the highest zone of Inhibition was Ciprofloxacin (25.5mm) while the lowest zone of inhibition was seen in Gentamycin (16.0 mm).

Table 4.6 showed that sample C (pasteurized coconut juice) has the highest Sodium content (1.32±0.009), followed by sample A, E, F then B while the minimum content was found in sample D (unpasteurized coconut juice) with the value (0.52±0.009), Sample A (pasteurized coconut + orange) has the maximum content of Magnesium

(1.13±0.092), followed by sample D, C, E, while the lowest content was seen in sample B (unpasteurized coconut + orange juice) and F (unpasteurized orange juice) with the value (0.13±0.025) and (0.13±0.139). The Calcium content ranges from 1.62±0.016 to 0.45±0.012 (ppm). The maximum content was found in sample A (pasteurized coconut + orange juice) with the value (1.62±0.016) and the minimum in sample D (unpasteurized coconut juice) with the value (0.45±0.012).

Discussions

Sensory properties of juice have a great importance to evaluate consumer attitudes and their influence on food choice and acceptability. The obtained mean panel score and statistical analysis showed that, juice of sample A (50:50 percent of orange extract and coconut extract (Pasteurized) is characterized with its highest score in all the parameters (color, taste, flavor, appearance and overall acceptability) and it was generally accepted, this is in line with the findings of Ogunmefun *et al*. (2018) followed by sample C, E and D. Sample A secured the highest score 7.7 and ranked as like moderately while sample D secured the lowest score of 7.2 and ranked as like slightly. In case of flavor preference, sample A is the most acceptable among six samples. Sample A secured the highest score 7.7 with moderately like ranking, followed by sample C (score = 7.2) and the lowest score was obtained by sample E (score = 7.1) in case of flavor. From Table 4.1b, it has been seen that sample A got the highest score of 7.7. In terms of overall acceptability, sample A, C and F were equally acceptable but sample D acceptability differs greatly. Thus, from sensory analysis it was discovered that Formulated mixed fruit juice blend containing 50% coconut juice and 50% orange juice secured the highest score color, flavor, sweetness and overall acceptability.

The pH is inversely proportional to the acidity. pH for all samples at various storage period was observed and shown in Table 4.2, the result showed that after three weeks of storage at room temperature the pH of samples examined were decreasing as week was increasing. The overall range of pH was 3.85 - 7.70. The highest value of pH in the third week was recorded for sample F and the lowest was recorded for sample C. The result of this research work deviated from the findings of Oke *et al*.(2010) who reported that the formulated mixed fruit juices was observe to be basic when stored for an elongated period of time. Kareem and Adebowale (2007) reported that the

dominant acid in orange juice is citric acid. Food acids dictate the dominant microflora in foods and to a large extent will determine the shelf stability of the juice (Ezeama, 2007). The more acidic the juice, the less susceptible to bacterial action but the more susceptible to the action of yeasts and moulds (Jay, 2000). Moreover, Anvoh *et al*. (2009) reported that fruit acids influence colour, flavour and gustative characteristics of the juice products. The acidity might be increased due to degradation of carbohydrates present in mixed fruit juice by the action of microorganisms. Increase in titratable acidity during the storage period may be due to activity of some acid producing bacteria such as *Alicyclbacillus acidosterrestris* (Sheikh, 2004). The present result was decreasing in acidity which may implies low microbial activities on the samples. The decrease in pH during storage might be due to degradation of carbohydrates present in mixed fruit juice by the action of micro-organisms.

The microbial loads for the fruit juice samples are shown and observed that there was an increase in the microbial load of the samples on storage which can lead to the spoilage of the fruit juice samples. These results conform to those obtained by Nwachukwu *et al*. (2008), Farzana *et al*. (2011), Oranusi *et al.* (2012) who recorded microbial load in fruits in the range of $10^{-4} – 10^{-9}$ cfu/ml. A total of 4 different bacteria species were isolated from the fruits juice samples analysed and they are *Bacillus megaterium, Bacillus polymx, Flavobacterium aquatile* and *Bacillus cereus*. The isolation of these organisms is supported by the work of Eni *et al*. (2010); Jolaoso *et al*. (2010) who isolated *B. cereus, Klebsiella sp, Salmonella, Escherichia coli* from fruits. Daniyan and Ajibo (2011) also isolated *S. aureus, S. epidermidis, Bacillus* sp*., E. coli*

and *Enterobacter aerogenes* from sliced fruits sold in Minna metropolis. This is further supported by the work of Oranusi and Olurunfemi (2011) who isolated *Bacillus* Sp., *S. aureus, E. coli, Enterobacter, Salmonella, Klebsiella, P. aeruginosa, Proteus, Micrococcus* and *Lactobacillus sp.* from fruits juice sold in Ota, Ogun State. This is also in line with the findings of Ogunmefun *et al.* (2018) which also isolated *Bacillus cereus* from unpasteurized orange juice. The acid and sugar content are critical to the survival of microbes and will ultimately affect the shelf life and sensory quality of the fruit juices samples (Jay, 2000; Ezeama, 2007).

The various biochemical tests such as Gram reaction, Cell morphology, catalase, Oxidase, Casein Hydrolysis, Gelatin Hydrolysis, Methyl red, Nitrate reduction, coagulase, Urease etc. tested on the samples revealed that majority of the isolates were

positive to the tests while some reacted negatively to the tests. The result also shows the growth of the isolate at different pH and temperature, there was growth in some such as *Bacillus megaterium* while there was no growth in some; this is in line with the result of Adesokan *et al.* (2009) who also observed a positive reaction with *Bacillus cereus* when subjected to these biochemical tests. All the organisms reavealed positive and negative reaction to sugar fermentation like glucose, Fructose, Maltose, Lactose, Sucrose, galactose etc. Oke *et al.* (2006) reported the positive reaction of *Flavobacteriun aquantile* to Xylose, Galactose, Maltose, and Fructose.

The result of the antimicrobial sensitivity test showed that many of the bacteria revealed an intermediate to sensitive reaction to most of the antibiotics tested. Some of the gram-positive bacteria were sensitive to Gentamycin and Ofloxacin. They showed no reaction to Erythromycin, Cefuroxime, Ceftazidime and Ceftriaxone except for *Flavobacterium aquantile* which was sensitive to it. These findings were supported by the works of Daniyan and Ajibo (2011) who reported that *B. cereus* was resistant to Pefloxacin but susceptible to Streptomycin, Ciprofloxacin, Ceftriaxone and Cefuroxime in their study. Srinu *et al*. (2012) also reported that *Staphylococcus aureus* was sensitive to Streptomycin, Clotrimoxazole and Ciprofloxacin. Agwa *et al*. (2012) found that *Bacillus cereus* was susceptible to Erythromycin, Ciprofloxacin and Streptomycin but resistant to Ampicillin, although our findings showed that *Bacillus cereus* was resistant to Ampicillin and Erythromycin. The present observation is in agreement with the study reported by Kumar *et al*. (2009) using antibiotics on test organisms with *C. limonum* juice extract which showed high susceptibility rate of several test organisms to Gentamycin and Ofloxacin.

Minerals play a key role in various physiological functions of the body, especially in the building and regulation processes. Fruits are considered as a good source of dietary minerals (Ismail *et al*., 2011). The lowest values of minerals obtained from this research compared to RDA are in accordance with the findings of Kumar *et al.* (2009), Evans and Halliwell, 2001, Janick, 2010 but were lower than the values reported by Anhwange *et al*. (2009), Manzoor *et al*. (2012) and Hussain (1985). According to Leterme *et al.* (2006), several factors like variety, state of ripeness, soil type, soil condition, and irrigation regime may cause variation in the mineral and trace elemental contents in different types of fruits as well as within different parts of the same fruit. Comparative study of the difference in value of the pasteurized sample and unpasteurized samples for sodium revealed that the pasteurized orange and coconut

mixtures has the highest values of Na and Ca while Mg values were low for all groups compared to RDA values. Similar observation was reported by Mansour *et al*. (2012).

Conclusion

Fruits both in fresh as well as in processed form not only improve the quality of our diet but also provide essential ingredients like vitamins, minerals, carbohydrates etc. It can be concluded that a fruit juice blend prepared from coconut and orange in a ratio of 50:50 percent can serve as homemade fruit juice which are better in terms of sensory parameters to the industrially packaged mixed fruit juices in the market, it also have positive health benefits. Consumer acceptability and storage stability of three samples (pasteurized mixed fruit juices, pasteurized orange juices and pasteurized coconut juices) implied that mixed fruit juice can be prepared by using a combination of 50% orange juice and 50% coconut juice with best consumer acceptability and can be kept up to a one month without any spoilage.

Recommendation

It can be recommended that good sanitary practices should be employed in the production of home-made fruit juice and commercially produced juices. From this study, antibiotics such as Ciprofloxacin, Ofloxacin, Ceftriaxone, Cefuroxime, Gentamycin, Erythromycin and Nitrofuratoin which inhibit the growth of the isolates can be used as remedy when there is consumption of contaminated fruit juice.

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