Determination of Proximate Composition and Microbial Contamination of Fresh Juice from Three Citrus Species

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Abstract

Research on the proximate composition and microbial contamination of fresh citrus juice from sweet orange (Citrus sinensis), lemon (Citrus limon), and lime (Citrus limonia) were conducted in the Microbiology laboratory in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt. The fresh squeezed juice was exposed for 24 hours for bacterial and 7 days for fungal contamination. Analysis of proximate composition showed that lemon (sour orange) had the highest values for moisture; lipid and Vitamin C content (85.1±0.005, 1.15±0.002 and 130.5±0.004) while sweet orange recorded the highest values for ash, protein and vitamin A. However, lime recorded the highest value for carbohydrate (23.08±0.002). Anti nutrient element (Tannins) was present in sweet orange and lemon but absent in lime. The total bacterial count from sweet orange juice ranged between 4.0 and 6.0 $x10^3$ cfu/ml; lemon juice ranged between 4.0 and 5.0 $x10^3$ cfu/ml while lime juice recorded growth range between 3.0 and 5.0 $x10^3$ cfu/ml. However, mixed citrus juice had a growth range between 3.0 and 4.0 x 10^3cfu/ml while total fungal count from sweet orange ranged between 3.0 and 6.0 $x10^3$sfu/ml; lemon and lime juices ranged between 4.0 and 5.0 $x10^3$ sfu/ml respectively while mixed fruit juice had a total fungal count which ranged between 4.0 and 7.0 $x10^3$ sfu/ml. Bacterial load was greatly reduced in the mixed citrus fruit juice while fungal total count was highest in the mixed fruit juice than in sweet orange, lime and lemon juice. Bacteria and fungi isolated from fresh citrus juice include; Bacillus sp, Lactobacillus sp, and Staphylococcus sp while fungal species include; Aspergillus niger, Fusarium oxysporum, Penicillium digitatum, Rhizopus delemar and Mucor vouxii. The result revealed that most of the samples had high microbial load, especially the mixed juice and sweet orange juice which indicated that they were heavily contaminated due to poor handling, poor sanitation and method of extraction.

Keywords: *Microbial, contamination, sweet orange, lime, lemon.*

Introduction

Citrus comprising Sweet orange, Lime and lemon are grown in warm climate. They belong to the kingdom *Plantae* and the family *Rutaceae (*Purseglove, *1977).* Citrus is the most important fruit tree crop in the world (Spiege-Roy et *al, 1996).* In addition to commercial production, it is widely grown for personal use in "indoor yards", roadsides, and small subsistence plots. Growing citrus for this use is extremely variable. The majority of citrus that are taken to the market are in the form of processed products such as orange juice and frozen juice concentrates. The distinct bright colour of citrus fresh fruit shows that in addition to

being beneficial for healthy eyes, lungs and skin, they are also rich in wide varieties of nutrients that help prevent different kinds of disease conditions. (Rinzler *et al, 1999).*

Citrus juice is known for their high vitamin C concentration, a nutrient that offers antioxidant and immune support (Rinzler *et al, 1999*, Achinewhu, 1996). They are nutritive, invigorative and non alcoholic beverage, which is very well liked throughout the world. Citrus are very palatable and a good source of foliate, thiamine, potassium, vitamin A, calcium, magnesium, phosphorus, sodium, and also a good source of fibre. (Ohiokpehai, 2003). Recently, the fresh squeezed citrus juice has increased, as unpasteurised juices are preferred by consumers due to their fresh flavour attributes. They are prepared by simply extracting the liquid and pulp of mature fruits either manually or mechanically, which gives a final product of an unfermented, clouded, untreated juice ready for consumption. Such juice has little or no processed steps that reduce pathogen level. Citrus juices are acidic beverage with high sugar content, under these conditions, acidolactic bacteria; moulds and yeast comprise the typical microbiota present in citrus juices. Lactic acid bacteria are the primary spoilage bacteria in fruit juices. However, economic losses due to juice contamination are minimized by good sanitation during and after processing. Pasteurization, concentration or low temperature storage protocols help to reduce the number of micro organisms in the final product. However, these products are not free from microbiological spoilage, especially non-pasteurized single-strength juices. (Okaka, 1997, Ihekoronye and Ngoddy, 1985). In most countries where street vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases associated with the street vended foods. However, microbial studies on such foods in countries like America, Asia have revealed increased bacterial pathogens in the foods. (Sandeep, *et al, 2004).* Citrus production in the tropics especially in Nigeria is on the increase as many people are involve in its production. Like all other perishables, it does not store very well except when processed in some forms. So much wastage has been recorded during the flush seasons as a result of microbial deterioration (Achinewhu, 1996). This work therefore, aims at investigating the proximate composition of fresh citrus juice from, sweet orange, lime and lemon and the microorganisms associated with their spoilage. The public health implications will also be highlighted.

Materials and Methods.

Collection of citrus samples

One basket full of sweet orange, lime and lemon was purchased from the mile 3 market in Port Harcourt and transported by a taxi to the Department of Applied and Environmental Biology Laboratory in the Rivers State University of Science and Technology for further studies

Method of juice extraction

The citrus fruits were sorted and the bad ones discarded. The fresh citrus fruits were washed in tap water and cut with a clean kitchen knife and squeezed individually into separate 2 litre sterile glass bowl and labelled accordingly. 10 millilitres from each of the juice samples was measured into another 2 litres glass bowl and also labelled which constituted the mixed citrus juice sample. The 4 glass bowls with their contents were exposed for 24 hours for bacterial contamination and 7 days for fungal contamination; Samples were also collected for proximate composition analysis.

Proximate composition determination

Fresh fruits of sweet oranges, lemon, and lime were purchased from the mile 3 market in Diobu, Port Harcourt and taken to the Food Science and Technology Laboratory in Rivers State University of Science and Technology for Proximate Analysis. The parameters estimated were moisture, ash, lipid, fibre, carbohydrate, protein, vitamins A and C, Tannins, iron, sodium, magnesium, phosphorus and Potassium. These parameters were analysed using the Association of Official Analytical method (AOAC, 1990) method.

Microbiological Analysis

Enumeration of heterotrophic bacteria and saprophytic fungi

The method used was the ten-fold serial dilution method of Harrigan and McCance (1990). Decimal dilutions of the samples were made by adding 1ml of fresh citrus juice samples to 9.0ml of sterile saline to give an initial dilution of 1:10. Subsequent serial dilutions were made by adding 1.0ml of the last dilution to 9.0ml of fresh diluents. Finally, 0.1ml of appropriate dilutions was inoculated using the spread plate technique onto Nutrient and Sabouraud Dextrose agar plates for heterotrophic bacteria and saprophytic fungi respectively. The agar plates were inverted and incubated at 37^{o}C for 24 hours for bacterial growth and for fungal growth, incubation lasted for 5-7 days. After incubation, numbers of colonies were counted from plates which yielded growth between 30 - 300 colonies.

Identification of Microbial isolates

Identification of bacterial isolates was based on methods of Buchanan and Gibbons (1974), Cowan (1974, and Cruickshank *et al.* (1975). These tests included Gram reaction, motility, catalase, oxidation/fermentation, hydrogen sulphide production, coagulase, oxidase, indole production, Methyl red-VogesProskauer (MR-VP) reactions, starch hydrolysis, nitrate reduction, and fermentation of the following carbohydrates, glucose, lactose, mannitol, and arabinose while fungal isolates were identified based on macroscopic and microscopic appearances, according to Alexopoulous, (1962) and Barnett *et al.* (1983).

Results

Results of the proximate composition of the three citrus species comprising the sweet orange, lemon and lime are presented in Table 1 below. Lemon (sour orange) recorded the highest values for moisture; lipid and Vitamin C content (85.1±0.005, 1.15±0.002 and 130.5±0.004) while sweet orange recorded the highest values for ash, protein and vitamin A. However, lime recorded the highest value for carbohydrate (23.08±0.002). Anti nutrient element (Tannins) was present in sweet orange and lemon but absent in lime.

Table 1: Proximate, vitamins, anti nutrient and mineral composition of sweet orange, lemon and lime.

Proximate composition (%)	**Samples**		
	Sweet orange	**Lemon**	**Lime**
Moisture	80.5±0.006	85.1±0.005	75.64±0.006
Ash	0.60±0.003	0.50±0.004	0.15±0.004
Lipid	0.25±0.005	1.15±0.002	0.05±0.003
Fibre	2.5±0.001	1.52±0.002	0.05±0.001
CHO	14.56±0.002	10.61±0.010	23.05±0.002

Protein	1.56±0.001	1.2±0.005	1.03±0.001
Vitamins			
Vitamin A (IU)	500±0.002	300±0.001	21.0±0.004
Vitamin C(mg)	125.4±0.001	130.5±0.004	87.9±0.001
Anti nutrient			
Tannins	0.32±0.004	0.40±0.011	ND-
Mineral Assay (%)			
Fe	0.4±0.003	0.45±0.005	0.5±0.004
Na	0.5±0.004	0.48±0.003	0.25±0.002
Mg	0.05±0.006	0.15±0.006	1.25±0.005
P	21.5±0.021	22.0±0.003	20±0.022
K	0.35±0.003	0.30±0.001	1.50±0.003

ND – Not Done

The results of the total heterotrophic bacterial count from the sweet orange, lemon, lime and mixed citrus juices are indicated in Figure 1 below. The total bacterial count from sweet orange juice ranged between 4.0 and 6.0 $x10^3$ cfu/ml; lemon juice ranged between 4.0 and 5.0 $x10^3$ cfu/ml while lime juice recorded growth range between 3.0 and 5.0 $x10^3$ cfu/ml However, mixed citrus juice had a growth range between 3.0 and 4.0 x 10^3cfu/ml. The implication of this is that mixed citrus juice showed low bacterial growth as against the high growth rate recorded for individual citrus juice. Sweet orange juice harboured more colonies of bacteria, followed by lemon and lime juices respectively.

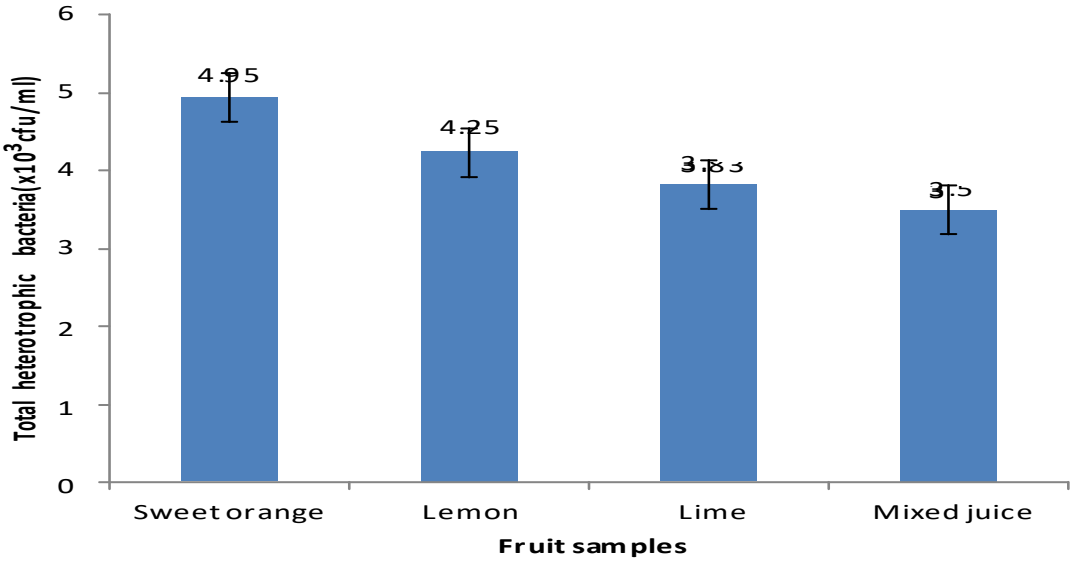

Figure 1: Mean Total heterotrophic bacterial count ($x10^3$cfu/ml) of sweet orange juice, lemon, lime and mixed juice samples

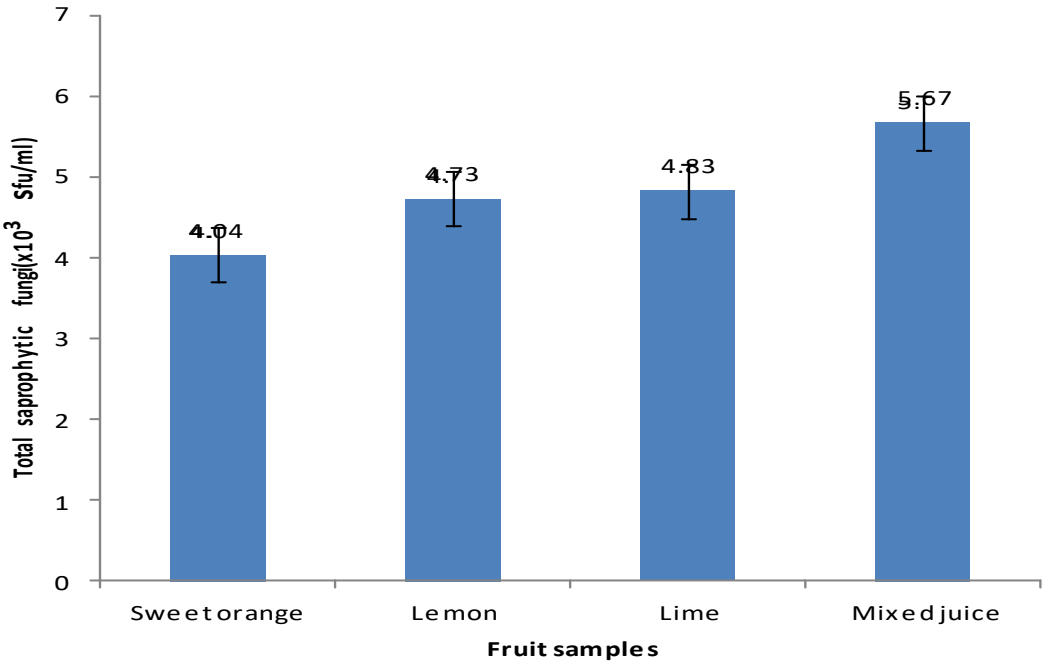

Figure 2: Mean Total saprophytic fungal count (x10^{3}sfu/ml) of sweet orange juice, lemon, lime and mixed juice samples

Results of total saprophytic fungal counts presented in Figure 2 above showed that counts from sweet orange ranged between 3.0 and 6.0 x10^{3}cfu/ml; lemon and lime juices ranged between 4.0 and 5.0 x10^3 cfu/ml respectively while mixed fruit juice had a total fungal count which ranged between 4.0 and 7.0 x10^3 cfu/ml This result showed that fungal contamination of mixed fruit juice was higher than what was recorded for sweet orange juice, lime and lemon as against the results obtained for bacterial contamination where the mixed fruit juice harboured the least bacterial count.

Bacterial and fungal isolates from sweet orange juice, lime, lemon and mixed fruit juice.

Results based on all the bacterial and fungal identification tests carried out revealed the following bacteria and fungi to be associated with citrus fruits juice contamination. They are *Bacillus sp, Lactobacillus sp* and *Staphyloccocus sp* (Bacteria) and *Aspergillus niger, Fusarium sp, Penicillium digitatum, Rhizopus delemar and Muccor vouxii (fungi).*

Discussion.

Fresh citrus juices are highly nutritious, invigorating and non alcoholic beverages which are liked throughout the world. Based on the result of the proximate composition, it has been discovered that the fresh juice from citrus contains high amount of carbohydrate, protein, vitamins A and C and other essential mineral elements. The high nutrient qualities of most tropical fruits, vegetables, nuts and herbs have been reported. This is why it is advised that more of these fruits and vegetables should be included in the diets. The high vitamins A and C values of these fruits further buttresses this point as has been reported by early researchers

(Achinewhu, 1996, Chuku *et al*, 2007, Chuku, 2010, 2012, and 2013). Most of the juice related outbreak occurs through contamination by pathogenic microorganisms (Ohiokpehi, 2003). Following the analysis of fresh citrus juice in this study, it was discovered that all the juice samples were contaminated with bacteria and fungi. The bacterial isolates were *Bacillus sp, Staphylococcus sp and Lactobacillus sp*. While the fungal isolates were *Penicillium sp, Fusarium sp, Aspergillus species and Muccor sp*. Striking differences in microbial growth were found between each of the samples with a total bacterial count that ranged from $3.0x10^4$ to $6.0x10^3$cfu/ml. Bacterial count was lowest in the mixed citrus fruit juice indicating the fact that mixed citrus fruits had some antimicrobial effect on the bacteria load. The presence of *Bacillus sp* could also be from the equipment and one of the ingredients of citrus which is sugar (Banwart, 1989).*Bacillus and Staphylococcus* are gram positive bacteria that are associated with the normal *microbiota* of man(skin and nasal passages). They may have been contacted from poor sanitation, processing, packaging and exposure to air. (Okaka, 1997). *Lactobacillus sp* were also isolated from the fresh citrus juices due to its acid tolerant nature. They are carbon-dioxide tolerant and achieve high growth rates in the presence of high sugar contents. (Banwart, 1989).

A wide range of fungi are often associated with the spoilage of most fruits and vegetables amongst which are *Aspergillus species, Fusarium sp, Rhizopus sp and Muccor* species (Onuegbu, 2002, Chuku *et al* 2008, Chuku, 2012). Fungi thrive very well under acidic condition which was abundantly provided by the various citrus juices and therefore resulted in greater fungal load in the citrus juices (Espinel-Ingroff *et al, 1998)*. The results for total fungal count showed that saprophytic fungi ranged from $3.2x10^3$ sfu/ml to $5.1x10^2$ sfu/ml.

The isolation of *Rhizopus* spp, *Fusarium and Penicillium* could be due to contamination from the external environment through air and flies (Chuku *et al*, 2007, FAO,1979). *Muccor sp* may also have been contacted from dust from air. The analysis from mixed citrus juice showed differ Cfu/ml indicating a reduced growth of bacteria which could be due largely to competition for nutrient amongst the organisms. Total fungal count ranged from 3.0 to 7.0 $x10^3$ sfu/ml indicating that fungi had the highest number of growth in citrus juices which could be due to nutritional provisions which greatly enhanced their growth rate as reported by (Onuegbu, 2002).

Conclusion

High quality fresh citrus juice could be maintained through proper sanitation during and after processing to avoid microbial contamination and consumers, safety. Fresh citrus fruit juices are now processed in public places and along roadsides thereby exposing the juice to microbial contamination from the environment which causes a reduction in their quality. Proper enlightenment on food processing and handling will expose the danger associated with microbial contamination. Long exposure of citrus fruit juice should be avoided.

Recommendation

It is therefore recommended that extraction of citrus fruit juice should be carried out in a hygienic environment and that the extracted juice should be stored in the refrigerator to reduce the rate of microbial contamination. However, the use of heat sterilisation which will destroy most of the fungi and bacteria is hereby advocated.

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