IJRM
INTERNATIONAL JOURNAL OF RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals

Vol.:1, Issue:4

Research Article

Effect of Pasteurization Temperatures on Microbial Loads and Nutritional Qualities of Orange and Pineapple Juices Purchased from Makurdi Metropolis Benue State

IJRM
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HUMAN JOURNALS

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Keywords: Orange, Pineapple, Juices, Pasteurization, Microbial loads

ABSTRACT

Investigations were carried out to ascertain the effect of pasteurization temperatures on microbial loads and nutritional qualities of orange and pineapple juice purchased from Makurdi metropolis Benue state. Samples were randomly collected from five locations namely; Wadata, Akpehe, Wurukum, North Bank and High Level. Ten-fold serial dilutions were carried out on the juice samples. Using pour plate method, 1.0ml of dilutions taken from 10^{-3} and 10^{-4} were inoculated in duplicates on Nutrient agar (NA), eosin methylene blue agar, mannitol salt agar, Salmonella-Shigella agar, Mannitol salt agar, MacConkey agar and Potato Dextrose Agar (PDA). Inoculated plates were incubated at 37^{o}C for 24 hours, while PDA plates for isolation of fungi were incubated at room temperature (25^{o}C) for 3-5 days. Proximate analysis was carried out on samples using standard methods. Pasteurization using low-temperature holding (LTH) and high-temperature short time (HTST) were carried out to reduce contaminants. Bacteria associated with both juices were; *Streptococcus* spp, *Staphylococcus* spp, *Bacillus* spp, *Escherichia coli*, *Salmonella* spp and *Shigella* spp while fungi were; *Penicillium* spp, *Saccharomyces cerevisiae*, and *Aspergillus* spp. Before pasteurization total bacterial count (TBC) and total fungi count (TFC) ranged from 3.38-4.04x10^{5} CFU/ml and 3.46-4.39x10^{5}cfu/ml respectively in orange while in pineapple TBC and TFC ranged from 3.33-4.12x10^{5} CFU/ml and 3.49-4.19x10^{5}cfu/ml respectively. Moisture content ranged from 89.72-91.34%, protein 0.56-0.76%, fat 0.00-0.01, crude fibre 1.38-1.94%, ash 0.34-0.53%, carbohydrate 5.97-7.67%, vitamin C 31.42-43.98 mg/100ml and pH 4.49-4.74 in orange juice. Similarly, moisture content in pineapple juice ranged from 89.81-91.31%, protein 0.21-0.28%, fat 0.00%, crude fibre 1.20-1.28%, ash 0.17-0.23%, carbohydrate 6.92-8.52%, vitamin C 26.83-31.94 mg/100ml and pH 4.41-4.60. After pasteurization using (HTST) the mean count for bacteria and fungi were 3.47x10^{4} CFU/ml and 3.44x10^{4} CFU/ml respectively in orange while 3.16x10^{4} CFU/ml and 3.30x10^{4} CFU/ml were recorded in pineapple juice. Organisms isolated after pasteurization were *Bacillus* and *Aspergillus*. There is a significant difference ($p<0.01$) using pasteurization to treat both juices to reduce the microbial count. There is no significant difference ($p>0.01$) in proximate composition for vitamin C and pH after pasteurization. Microbiological quality of vended locally produced juices is very poor. Therefore, appropriate measures such as pasteurization should be carried out to improve the microbiological quality of juices.

INTRODUCTION

Fruit juices account for more than 90% of the total fruit production in Nigeria. The western part of Nigeria is the principal fruit juice producing region in the country (FAO, 2000). The major components of fruit and fruit juices are vitamin C and glucose (Livine *et al*., 2000, Shrubsole *et al.*, 2009). Juice may be prepared in the home from fresh fruits and vegetables using the variety of hand or electric juicers (FAO, 2002). There are reports of foodborne illness associated with the consumption of fruit juices at several places (Chumber *et al*., 2007). Pathogenic organisms can enter fruits and vegetables through damaged surfaces, such as punctures, wounds, cuts, and splits that occur during growing or harvesting (Durgesh *et al.,* 2008). Contamination from raw materials and equipment, additional processing conditions, improper handling, the prevalence of unhygienic conditions contribute substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables(Durgesh *et al.,* 2008). Water used for juice preparation can be a major source of microbial contaminants including fecal coliforms(Durgesh *et al.,* 2008). Fruits contain high levels of sugars and other nutrients, their low pH values make them particularly desirable to fungal decay(Al-Hindi *et al.,* 2011). Changes in pH may also promote the growth of pathogens (FDA, 2001). Due to the low pH of orange juice (pH <4), growth of pathogenic microorganisms is suppressed; Pasteurization of juice is designed to inactivate spoilage microorganisms (yeasts and lactic acid bacteria), conventional thermal processing often leads to detrimental changes in the sensory and nutritional qualities of such product (Vikram *et al*., 2005).

MATERIALS AND METHODS

Sample collection

A total of 50 locally produced fruit juice samples comprising of pineapple and orange were obtained randomly from five locations popularly known for vending fruit juices. The locations are; Akpehe, High level, Northbank, Wadata, and Wurukum. All the samples were collected in small transparent plastic bottles of 750ml as sold and transported in an ice-packed cooler to the laboratory, Department of Biological Sciences, the Federal University of Agriculture Makurdi for analyses.

Microbiological analysis of samples

Microbiological analysis such as isolation, enumeration, and identification of pathogens using standard procedures was employed. Media used for this analysis were the Oxoid brand of nutrient agar, peptone water, eosin methylene blue agar, mannitol salt agar, Salmonella-Shigella agar, Mannitol salt agar, MacConkey agar and Potato dextrose agar. All the media were prepared according to manufacturers' instruction. For the isolation and enumeration of pathogens in the samples, each fruit juice sample was shaken thoroughly to have a homogenized solution. Ten folds serial dilution was performed and 1.0ml of last two dilutions (10^{-4} and 10^{-5}) were inoculated in triplicate on appropriate media using pour plate technique. Bacterial plates were incubated at 37°C for 24 - 48hrs, while the fungal plates were incubated at room temperature (28°C)for 5 days. After incubation, both the bacterial and fungal plates were examined for the presence of discrete colonies. Colonies were counted and expressed as colony forming unit per milliliter (CFU/ml) of sample homogenate. Total aerobic counts were performed on nutrient agar, while *E.* coil and fungi were enumerated on eosin methylene blue agar and Potato dextrose agar respectively. Mannitol salt agar and MacConkey agar were used to enumerate *Staphylococcus aureus* and non-*E. coli* coliforms respectively, while *Salmonella- Shigella* agar was used for *Salmonella* counts after 24h pre-enrichment of sample homogenate in Selenite-F broth according to (Oranusi and Olorunfemi, 2012). Characteristic discrete colonies on the different media were isolated and purified by repeated sub-culturing on the same media. Pure colonies were stored on agar slants at 4°C for further characterization.

Identification of isolates

The method of Oranusi and Olorunfemi (2012) was employed for the identification of coliforms and aerobic bacteria while the fungal isolates were identified using the methods of Cheesbrough, (2005). The identities of coliforms and aerobic bacteria were confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology by Bergey and Holt (1994).

Total Heterotrophic Bacterial Count

After overnight incubation, growth on the Nutrient agar (NA) showing 30-300 colonies was counted. Bacterial counts were expressed as colony-forming-units per ml (CFU/ml) of fruit juice sample analyzed.

Total coliforms count

The technique used was counting viable growth on MacConkey agar to detect the presence of both total and fecal coliforms. The test was performed in the laboratory using aseptic techniques to avoid cross-contamination. The test was carried out in triplicate and counts expressed in (CFU/ml) colony forming unit per milliliter (Cheesbrough, 2005).

***Salmonella-Shigella* agar**

Sterile Salmonella- Shigella agar plates were used, by aseptically transferring 0.1ml aliquots from the ten-fold dilutions. These were incubated at 37^0C for 24 hours. Black colonies indicated Salmonella while pale pink colony indicated Shigella (Cheesbrough, 2005).

Total fungal count

The technique used was poured plate method. A sterile 1ml pipette was used to transfer 1.0ml of the juice sample into Petri dishes (in triplicate). Potato Dextrose agar was used to which 0.1ml of lactic acid was added to 100ml of cooled agar base at about 45^0C and stirred thoroughly. The plates were allowed to stay inverted and incubated at room temperature (25^0C) for 48 hours for yeast and 96 hours for molds, plates yielding counts of 30 -300 colonies were counted (Cheesbrough, 2005).

Microbial identification

Fungal colonies were characterized based on their cell or colonial morphological features (Cheesbrough, 2005). Bacterial isolates were identified following Gram reaction, spore staining and biochemical tests (indole, methyl red, citrate, catalase, coagulase, oxidase, urease, sugar fermentation) as described by Cheesbrough (2005).

Proximate Composition Analysis

Methods of AOAC (2005) was adopted in the determination of proximate analysis.

Determination of Vitamin-C

The method is applicable to the determination of reduced ascorbic acid. Ascorbic acid reduces oxidation-reduction indicator dye, 2, 6 - dichloroindophenol to a colorless solution. After the ascorbic acid was oxidized to dehydroascorbic acid, excess dye remains pink in acid

solution. At endpoint, the excess unreduced dye was rose pink in acid solution. Vitamin was extracted and titration performed in presence of HPO_3-CH_3COOH or HPO_3-CH_3COOH-H_2SO_4 solution to maintain proper acidity for reaction and to avoid auto-oxidation of ascorbic acid at high pH.

***Reagents*:** Ascorbic acid Standard Solution, 1 mg/mL, 2,6-Dichloroindophenol Standard Solution, Metaphosphoric acid-acetic acid solution, Sulfuric acid, 0.3 N, Metaphosphoric acid-acetic acid-sulfuric acid solution.

***Sample preparation*:** Test sample was weighted in 100 mL beaker and homogenized with approximately 50 ml HPO3.CH3COOH solution using tissue homogenizer until the sample was in suspension. Homogenized sample transferred into a 100 ml volumetric flask and diluted to volume with HPO3.CH3COOH solution.

***Blank test*:** seven ml HPO3.CH3COOH solution into a 50 mL Erlenmeyer flask was measured in triplicate and titrated with indophenol solution until rose pink color persist for 10 seconds. The volumes (ml) used was recorded and mean was calculated.

***Titration of the sample*:** Aliquots of 5 ml of sample was pipette out containing Ca^2 mg ascorbic acid into each of two 50 ml Erlenmeyer flasks. Enough HPO3.CH3COOH solution was added to make a total volume of 7 ml. Indophenol solution was titrated using a digital burette. The volume was recorded in ml.

Calculation of mg ascorbic acid per ml indophenols solution (Factor):

mg / ml Ascorbic acid standard = Weight Ascorbic acid standard / Total dilution volume

Factor = [{2 ml × mg/ml ascorbic acid standard} / {Mean vol. (mL) indophenols std. – Mean vol. (ml) blank}]

Calculation of mg ascorbic acid per 100 g sample: mg/100Vit C = [{(Vol. indophenol solution – blank) × factor × Total vol. × Dil. × 100} / (Weight × Aliquot)] (Bajaj and Gurdeep, 1981)

Pasteurization

Method 1: Low-temperature Holding (LTH)

Exactly 200ml of fruit juice was measured into a sterile beaker and placed in a water bath with automated temperature gauge with time. The juice samples were pasteurized at a temperature of 63 ± 2 °C for 30 minutes (D'Amico *et al*., 2006).

Method 2: High-temperature short time (HTST)

Two hundred (200) ml of fruit juice was measured into a sterile beaker and placed in a water bath with automated temperature gauge with time. The juice samples were pasteurized at a higher temperature of 90 ± 2 °C for 15 to 30 seconds (D'Amico *et al*., 2006).

RESULTS AND DISCUSSION

Table 1: Biochemical Characteristics of Bacterial Isolates

Colony code	Gram rxn	Shape	Catalase	Coagulase	Motility	Citrate	Indole	Methyl Red	Organism
PYCS	+	Rod	+	-	-	-	-	-	*Bacillus sp.*
PYCR	+	Cocci	+	+	-	-	-	-	*Staphylococcus aureus*
PRCS	-	Rod	-	-	+	-	-	-	*Shigella sp*.
PBCS	-	Rod	+	+	+	+	-	+	*Salmonella sp*.
PRCR	-	Rod	+	-	+	-	+	-	*Escherichia coli*
PBCR	+	Cocci	-	-	+	+	-	-	*Streptococcus sp.*

KEY: PYCS-pale yellow circular smooth, PYCR-pale yellow circular rough, PRCS-pink red circular smooth, PBCS- purple black circular smooth, PRCR-pink red circular rough, PBCR-purple black circular rough, rxn- Reaction, (+)- positive and (-)- negative

Table 2: Characteristics of Fungal Isolates

Colony code	Cultural characteristics	Morphological characteristics	Isolates
GCSR	Circular powdery green colony	Hyphae septate conidia attached to a vesicle via sterigma	*Penicillium sp.*
CDFS	Creamy flat colonies that are dull and dry	Oval budding cells/ gram +ve	*Saccharomyces cerevisiae*
GGWS	Green-gray colonies with a white apron at the margin	Long conidiophores and club-shaped vesicles	*Aspergillus sp.*
PYJB	Colonies start white to pale yellow but quickly form jet-black conidia	Profuse conidia with circumferential obscure vesicle	*Aspergillus sp.*

KEY: GCSR- green circular smooth and raised, CDFS- creamy dull flat and smooth, GGWS- green gray white and smooth, PYJB- pale yellow jet black and +ve –positive

Table 3: Microbial Loads of Orange Juice from Various Locations

Location	Coliforms ($x10^3$) (cfu/ml	Bacteria ($x10^5$) (cfu/ml)	Fungi ($x10^5$) (cfu/ml)
Wadata	*1.33^{a}	3.93^{a}	4.18^{ab}
Wurukum	1.21^{ab}	4.04^{a}	4.39^{a}
North Bank	1.19^{ab}	3.72^{ab}	3.77^{bc}
Akpehe	1.13^{b}	3.58^{ab}	3.65^{c}
High Level	1.11^{b}	3.38^{b}	3.46^{c}

*Values are means or triplicate readings. Means with different superscripts on the same column are significantly different by Duncan Multiple Range test

Table 4: Effect of Treatment on Microbial Loads of Orange Juice

Treatment	**Coliform** (cfu/ml)	**Bacteria**(cfu/ml)	**Fungi** (cfu/ml)
UNP	*3.59 x $10^{3\ a}$	3.71x $10^{5\ ab}$	3.97 x $10^{5\ a}$
PLH	0.00 x $10^{3\ b}$	4.01 x $10^{3\ a}$	4.26 x $10^{3\ a}$
PHH	0.00 x $10^{3\ b}$	3.47 x $10^{3\ b}$	3.44 x $10^{3\ b}$

*Values are means of triplicate readings. Means with the same superscripts on the same column are not significantly different by Duncan Multiple Range test.

Key: UNP- unpasteurized; PLH – Pasteurization with low heat; PHH – Pasteurization with high heat

Table 5: Effect of Pasteurization on Proximate Composition of Orange Juice

Treatment	Protein (%)	Fat(%)	Fibre(%)	Ash(%)	Moisture(%)	COH(%)	Vit.C mg/100ml	pH
UNP	*0.68^{a}	0.02^{a}	1.63^{a}	0.46^{a}	90.02^{b}	7.19^{a}	58.40^{a}	5.38^{a}
PLH	0.66^{a}	0.00^{b}	1.54^{a}	0.44^{a}	90.49^{ab}	6.83^{ab}	29.85^{b}	4.19^{b}
PHH	0.66^{a}	0.00^{b}	1.56^{a}	0.44^{a}	90.94^{a}	6.39^{b}	29.45^{b}	4.11^{b}

*Values are means of triplicate readings. Means with the same superscripts on the same column are not significantly different by Duncan Multiple Range test.

Key: UNP- unpasteurized; PLH – Pasteurization with low heat; PHH – Pasteurization with high heat; COH-carbohydrate

Table 6: Microbial Loads of Pineapple Juice from Various Locations

Location	**Coliform** (cfu/ml)	**Bacteria** (cfu/ml)	**Fungi** (cfu/ml)
Wadata	1.39 x $10^{3\ a}$	4.12 x $10^{5\ a}$	4.19 x $10^{5\ a}$
Wurukum	1.31 x $10^{3\ a}$	4.12 x $10^{5\ a}$	4.04 x $10^{5\ ab}$
Akpehe	1.14 x $10^{3\ b}$	3.89 x $10^{5\ b}$	3.61 x $10^{5\ c}$
Northbank	1.11 x $10^{3\ b}$	3.78 x $10^{5\ b}$	3.82 x $10^{5\ bc}$
High-level	1.08 x $10^{3\ b}$	3.33 x $10^{5\ c}$	3.49 x $10^{5\ c}$

*Values are means of triplicate readings. Means with the same superscripts on the same column are not significantly different by Duncan Multiple Range test.

Table 7: Effect Of Pasteurization on Microbial Loads of Pineapple Juice

Treatment	**Coliform** (cfu/ml)	**Bacteria** (cfu/ml)	**Fungi** (cfu/ml)
UPN	*3.61 x $10^{3\ a}$	5.09 x $10^{5\ a}$	4.61 x $10^{5\ a}$
PLH	0.00 x $10^{3\ b}$	3.30 x $10^{3\ b}$	3.58 x $10^{3\ b}$
PHH	0.00 x $10^{3\ b}$	3.16 x $10^{3\ c}$	3.30 x $10^{3\ c}$

*Values are means of triplicate readings. Means with the same superscripts on the same column are not significantly different by Duncan Multiple Range test.

Key: UNP- unpasteurized; PLH – Pasteurization with low heat; PHH – Pasteurization with high heat

Table 8: Effect of Pasteurization on Proximate Composition of Pineapple Juice

Treatment	Protein(%)	Fat(%)	C/Fibre(%)	Ash(%)	Moisture(%)	COH(%)	Vit.C mg/100ml	pH
UNP	0.27[a]	0.00[a]	1.23[a]	0.21[a]	90.52[a]	7.81[a]	37.61[a]	5.22[a]
PLH	0.25[b]	0.00[a]	1.22[a]	0.20[a]	90.72[a]	7.63[b]	25.10[b]	4.19[b]
PHH	0.24[b]	0.00[a]	1.21[a]	0.19[a]	90.72[a]	7.57[b]	24.24[b]	4.09[c]

*Values are means of triplicate readings. Means with the same superscripts on the same column are not significantly different by Duncan Multiple Range test.

Key: UNP- unpasteurized; PLH – Pasteurization with low heat; PHH – Pasteurization with high heat; COH-carbohydrate

DISCUSSION

In this study, microorganisms isolated from both juice samples from all locations include; *Bacillus* spp, *Salmonella* spp, *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, *Shigella* spp, *Penicillium* spp, *Aspergillus* spp and *Saccharomyces cerevisiae* as shown in(Tables 1 and 2). In contrast, Odu and Adeniji (2013) recorded that there were no coliform bacteria, *Salmonella* spp and *Shigella* spp isolated from juice samples and this difference could be due to the poor hygienic process used in production or handling. The occurrences of *Bacillus* sp. could be as a result of the prevalence of their spores in the environment (Amusa *et al.,* 2005). *Bacillus sp*ecies formed spore which could survive high temperatures of processing. Previous reports by Amusa and Asheye, (2009) showed that the presence of *E.coli* in hawked drinks is expected since the source of water used in many parts of Nigeria is tap and underground water, this is an indication of fecal contamination. Coliformshave has been reportedly associated with tap water popularly consumed in some towns in Nigeria

(Amusa and Asheye, 2009). In this study, the presence of *Staphylococcus species* in all samples could be as a result of contaminations from handlers. *Staphylococcus aureus*, a mesophile has been implicated in food poisoning outbreak of some food materials (Adubofuor*et al*., 2010).

The result obtained from this study showed that the total bacteria count of the locally produced fruit juice samples was very high (Tables3 and 6). High counts could be as a result of water used for processing and from handlers. High moisture content was recorded in both juice samples and this agrees with the report of Health and Reineccius(2006). Carbohydrate was higher in pineapple juice than in orange juice with mean values ranging from 6.92-8.52% and 5.97-7.67% respectively. This is in agreement with Nazarudeen(2010) who opined that orange was poor in protein, fat, crude fiber, ash, and carbohydrate. Pineapple juice had lower Vitamin C (26.83-31.94)mg/100ml values compared to orange juice (31.42-43.98)mg/100ml from all locations. The content of vitamin Cis an important parameter for assessing the nutritional value of the food as it degrades during pasteurization and storage (Franke *et al*., 2004). There was no significant difference ($p>0.05$) between proximate compositions in both fruits. It was established from this study that Pasteurization had a significant impact on both the microbial loads and some proximate components of the juice samples analyzed. Pasteurization using high temperature shorter time (HTST) recorded the lowest microbial counts in both juice samples analyzed (Tables 4,5.7 and 8). Pasteurization destroyed all non-spore forming organisms but *Bacillus* sp and *Aspergillus* sp survived because their spores could not be destroyed by the heat of Pasteurization. This is in agreement with the study carried out by Walls and Chuyata (2000). In this study both pasteurization methods had a significant effect on vitamin C and pH of the two juice samples analyzed (Tables 5 and 8). This is in agreement with a study carried out by Ogbonna *et al*. (2013) that recorded 49.45 (unpasteurized) and 21.56 (pasteurized). The effect could be due to the fact that vitamin C is a water-soluble vitamin and can easily be destroyed by heat.

CONCLUSION

It was concluded from the findings of this study that most orange and pineapple juices vended in Makurdi metropolis are heavily contaminated. These organisms; *Bacillus* sp, *Staphylococcus* spp., *Escherichiacoli*, *Shigella*spp., *Salmonella* spp., *Streptococcus* spp., *Penicillium* spp., *Aspergillus* spp. and *Saccharomyces* species were isolated from the locally produced juices. The average counts for bacteria and fungi of the locally produced fruit juice

samples examined were above the maximum allowable limit in fruits to be marketed for consumption (10^3cfu/mL). These high counts are suggestive of heavy bacterial contamination of these locally produced fruit juices and this can pose serious health problems. It has been established from this study that Pasteurization of juices using high-temperature short time (HTST) can reduce contaminants to a minimal level.

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