

# Effect of Pasteurization and Storage Temperatures on the Quality of Carrot Juices

<sup>1</sup>Daniel Enefiok Akpaetok, <sup>1</sup>Ihuoma Ahaotu and <sup>2</sup>Ndukwe Maduka

<sup>1</sup>Department of Microbiology, Faculty of Science, University of Port Harcourt, Choba, Rivers, Nigeria

<sup>2</sup>Department of Microbiology, Faculty of Science, Federal University Otuoke, Yenagoa, Bayelsa, Nigeria

## ABSTRACT

**Background and Objective:** Unstandardized preparation methods and poor storage conditions of homemade fruit and vegetable juices could impact the quality of the product. Therefore, this study seek to determine the effect of unpasteurization and room temperature storage associated with homemade carrot juice on its microbiological, physicochemical and sensorial properties.

**Materials and Methods:** Total heterotrophic bacterial count, total fungal count, pH, titratable acidity and total soluble solids of unpasteurized and pasteurized carrot juices stored at 0°C for 5 weeks; 4 and 24°C for 5 days were monitored at intervals using standard methods. Sensory evaluation of the samples involved the use of a 9-point hedonic scale. The data obtained were subjected to statistical analysis using a One-way Analysis of Variance (ANOVA) and probability value at  $p < 0.05$  was considered to be significant.

**Results:** Total heterotrophic bacterial count (THBC) of pasteurized carrot juice stored at 0 and 4°C was within the range of  $< 1.0-3.66 \log_{10} \text{CFU mL}^{-1}$ . The juices were within the THBC limits recommended by the International Commission on Microbiological Specifications of Food (ICMSF) and Codex Alimentarius Commission (CAC) of the Food and Agricultural Organization (FAO). The total fungal count of carrot juices stored at 4 and 24°C was  $< 1.0 \log_{10} \text{CFU mL}^{-1}$  with the exception of the unpasteurized juice stored at 24°C. During storage, the titratable acidity of the carrot juices increased from 0.3-2.8% whereas, the total soluble solids (6-23 °Brix) and pH (3.4-6.3) decreased with few exceptions. Based on sensory report, the pasteurized carrot juice stored at different temperatures was preferable than the unpasteurized juice.

**Conclusion:** Personal and environmental hygiene, pasteurization and low temperature storage of homemade carrot juice are recommended to ensure that the products are good quality and microbiologically safe for human consumption.

## KEYWORDS

Foodborne illnesses, shelf life, pasteurization, non-communicable diseases, healthy diets, carrot juice

*Copyright © 2023 Akpaetok et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.*

## INTRODUCTION

In recent years, the lifestyle of millions of people in terms of feeding habits has been influenced by globalization. Consequently, the number of cases of obesity, cardiovascular diseases, chronic respiratory diseases, cancer and diabetes have been on the increase. The World Health Organization (WHO) is vigorously promoting the consumption of healthy diets by recommending everyone to eat a minimum of 400 g of fruits and vegetables daily. According to statistics published by the WHO, the estimated



number of deaths that occurred globally in 2013 as a result of consuming little quantity of fruits and vegetables is 6.7 million<sup>1,2</sup>. According to Pem and Jeewon<sup>3</sup>, adequate consumption of fruits and vegetables significantly reduces the risk of non-communicable diseases and helps to control obesity.

Fruits and vegetables are well-known to be rich in different types of vitamins (A, B, C and E) and minerals (Na, K, Mg, S, Ca, Mn, Cu, Fe, Zn and Cu) required by the body in small quantities to carry out metabolism and other normal functions<sup>4,5</sup>. Carrot (*Daucus carota* L.) is a root vegetable cultivated in all parts of the world. It is a member of the family Apiaceae<sup>6</sup>. Based on its pigmentation, carrots cultivated for human consumption are divided into two groups: Eastern carrots and Western carrots. There is no tentative information on the origin of western carrots, but eastern carrots have been traced to Afghanistan and Pakistan. According to scientists, carrots originated in both countries about 1,100 years ago<sup>7,8</sup>. There are several varieties of carrots easily identified based on colours. They include black, purple, red, yellow and white carrots<sup>9</sup>.

In terms of nutritional composition, carrots occupy the 10th position out of 39 fruits and vegetables evaluated<sup>10,11</sup>. Carrot is popular because it is rich in  $\beta$ -carotene which is a precursor for vitamin A<sup>12,13</sup>. The proximate composition of carrots reported by Ahmad *et al.*<sup>14</sup> shows that it contains moisture (84-95%), protein (0.6-2.0%), fat (0.2-0.7%), carbohydrates (9.58-10.6%) and fiber (0.6-2.9%). Carrots also contain vitamin C (5.9 mg/100 g), vitamin B<sub>1</sub> (0.07 mg/100 g), vitamin B<sub>2</sub> (0.06 mg/100 g), vitamin B<sub>3</sub> (0.93 mg/100 g), vitamin B<sub>6</sub> (0.138 mg/100 g),  $\beta$ -carotene (8285  $\mu$ g/100 g),  $\alpha$ -carotene (3477  $\mu$ g/100 g) and vitamin E (0.66 mg/100 g). Minerals such as magnesium, manganese and potassium are found in reasonable amounts in carrots. Some of the minerals present in carrots help to fight tooth decay and cavities. Molybdenum is rarely found in many vegetables, however, it is present in carrots. Molybdenum is a trace mineral that helps the body to metabolize fats and carbohydrates as well as absorb iron<sup>4,13</sup>.

The presence of phytonutrients which include phenolics, polyacetylenes and carotenoids in reasonable amounts in carrots is a promoter of good health in humans<sup>15</sup>. Several health benefits associated with eating carrots regularly include fast healing of wounds, useful in fighting diabetes, cancer, hypertension, cardiac disease and reduction in the level of cholesterol in the body. Extracts from the seed possess antibacterial, antifungal, analgesic, cardio and hepatoprotective properties. Originally, the use of carrots was basically because of their medicinal properties which later became very useful as food for human consumption<sup>9,16</sup>.

In addition to chewing carrots either in raw or cooked form, it can be processed into various products which include powder, juice, candy, beverages, chips, canned carrots, kheer and halwa. Preparation of soups, beverages, curries, wine, jams, stews and pies could be enhanced by incorporating carrots<sup>5,14</sup>. Consumption of carrot juice helps to flush the kidney. It is also helpful in relieving congestion or inflammation of the kidney. Daily consumption of a cup of carrot juice helps to improve eyesight, skin, blood sugar level and healthiness of the blood. Since  $\beta$ -carotene is a precursor of vitamin A, carrot juice helps to protect the eyes and skin; fight against free radicals which cause damage in the body leading to the development of chronic diseases (heart disease and cancer); helps boost the functions of the brain, immune system and cognitive function. It also helps in reducing the risk of dementia and conditions associated with memory loss. Carrot juice is helpful for lactating mothers because it enriches breast milk with nutrients for the baby<sup>1,13</sup>.

A lot of people enjoy drinking unpasteurized vegetable and fruit juice because it is fresh, rich in vitamins and the calorie content is low<sup>17</sup>. Due to increasing awareness about healthy lifestyles and diets, an increasing number of consumers of fruit and vegetable juice prefer the product prepared at home without adding preservatives to commercially packed juices that contain chemical preservatives. Without

pasteurization, ready-to-drink vegetable and fruit juice is likely to contain a high microbial load including potentially harmful microorganisms. Therefore, the product will have a short shelf life. A study carried out by Adeleke *et al.*<sup>18</sup> reported that carrots sold in some markets were contaminated with *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Citrobacter* sp. and *Candida* sp. The consumption of unpasteurized carrot juice prepared using carrots bought from the market could lead to food-borne illnesses. Most people's immune systems can fight off the symptoms of certain food-borne illnesses. However, children, the elderly and immunocompromised individuals are at risk of becoming ill as a result of drinking unpasteurized carrot juice. Therefore, this study is aimed at determining the effect of pasteurization on microbiological, physicochemical and sensorial properties of carrot juice stored at 0°C, 4°C and ambient temperature (24°C).

## MATERIALS AND METHODS

**Study area:** The study was carried out in the Microbiology Laboratory, University of Port Harcourt, Choba, Rivers State. The duration of the study is 6 weeks; between May to June, 2023.

**Preparation of carrot juice:** About 14 kg of Nantes variety of carrots (*Daucus carota* sp., *sativa*) were bought from a local vegetable fruit garden market in D-line, Port Harcourt using a sterile polyethene bag. Before processing the carrots, they were washed with potable water. Thereafter, the head and bottom of each carrot were cut off followed by peeling the bark with a clean stainless steel knife sterilized with 70% ethanol. A repeat washing of the peeled carrots was done. The clean peeled carrots were diced (20×20×20 mm) and soaked in potable water for 30 min. The diced carrot (12 kg) was macerated with 12 L of potable water and the carrot juice was extracted using a juice extractor (Kenwood Accent Collection Juicer, JEM02). The carrot juice was poured inside a sterile universal sample container (30 mL). A total of 90 universal sample containers were filled with carrot juice and tightly corked. Half of the total number of containers containing carrot juice (45 samples) were pasteurized using the low-temperature long time method (63°C for 30 min) whereas the remaining half of the containers (45 samples) were unpasteurized.

**Storage of carrot juice:** Immediately after pasteurization was completed, both pasteurized and unpasteurized carrot juice samples were stored at 0, 4 and 24°C (ambient temperature). Samples of unpasteurized and pasteurized samples stored at 24°C and 4°C were withdrawn daily for microbiological and physicochemical analysis. Similarly, samples from the unpasteurized and pasteurized samples stored at 0°C were withdrawn at 1 week interval for microbiological analysis.

**Serial dilution:** A 10-fold serial dilution for each sample of carrot juice was carried out using sterile buffered peptone water. Exactly 10 mL of carrot juice sample was diluted with 90 mL of 0.1 % sterile peptone water. Serial dilution upto 10<sup>-5</sup> was done by transferring 1 mL of the stock solution into subsequent test tubes containing 9 mL sterile peptone water using a sterile pipette for each transfer.

**Determination of total aerobic plate count:** Dilutions of 10<sup>-3</sup> and 10<sup>-5</sup> for each sample of carrot juice was surface spread onto duplicate sterile nutrient agar (NA) plates for enumeration of bacteria. The inoculated plates were incubated at 37°C for 24 hrs. Uninoculated NA plate served as the control. After incubation, discreet colonies on NA plates were manually counted and recorded. The formula below described by Ahaotu *et al.*<sup>19</sup> was used to calculate the aerobic plate count expressed as Colony Forming Units per mL (CFU mL<sup>-1</sup>):

$$\text{CFU mL}^{-1} = \text{Number of colonies} \times \frac{1}{\text{Dilution factor}} \times \frac{1}{\text{Volume plated}}$$

**Determination of total fungal count:** Exactly 1 mL solution from dilutions  $10^{-3}$  and  $10^{-5}$  were surface spread onto duplicate potato dextrose agar (PDA) plates supplemented with antibiotics for enumeration of fungi. The inoculated agar plates were incubated at  $28 \pm 2^\circ\text{C}$  for 5 days. Uninoculated PDA plate served as the control. After incubation, discrete colonies on PDA plates were manually counted and recorded. The formula below was used to calculate the total fungal count expressed as Colony Forming Units per mL ( $\text{CFU mL}^{-1}$ ):

$$\text{CFU mL}^{-1} = \text{Number of colonies} \times \frac{1}{\text{Dilution factor}} \times \frac{1}{\text{Volume plated}}$$

**Obtaining pure cultures:** Distinct colonies were randomly collected using a sterile wire loop and sub-cultured on freshly prepared sterile NA plates and incubated for 24 hrs at  $37^\circ\text{C}$  for bacterial isolates. A similar procedure was carried out for fungal isolates which were sub-cultured on freshly prepared sterile PDA plates and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 5 days to obtain pure cultures.

**Identification of bacterial isolates:** Bacterial isolates were identified based on microscopic, colonial morphology and biochemical characteristics with reference to Bergey and Holt<sup>20</sup> and Shoaib *et al.*<sup>21</sup>. Gram staining and motility test of the bacterial isolates was performed. Biochemical tests of each bacterial isolates involved catalase test, oxidase test, citrate test, indole test, sulphur test, sugar fermentation, triple sugar iron test and Methyl-Red-Voges-Proskauer test<sup>21,22</sup>.

**Identification of fungi isolates:** Pure fungal isolates were placed on clean and grease free slide and a drop of lactophenol was added. The preparation was covered with coverslip. Immediately, the preparation was observed under  $\times 10$  and  $\times 40$  objective lenses. Identification of the fungi isolates was done by comparison of observed features with a fungi atlas<sup>23</sup>.

#### Physicochemical analysis

**pH:** The pH of carrot juice sample was measured at room temperature ( $28 \pm 2^\circ\text{C}$ ) using a digital pH meter (Thermo Orion, 710A+). A standard buffer solution prepared with 1.23 g of boric acid (0.2 N) and 1.49 g of potassium chloride in 100 mL of distilled water was used as solution for standard pH. Carrot juice was poured inside a graduated cylinder until it reached 30 mL mark. The probe of the pH meter was dipped inside the carrot juice sample for 3 min. When the figure on the display was stable, the pH reading was taken. The measurement was carried out in duplicate for each sample.

**Total soluble solids (TSS):** The method described by Kong *et al.*<sup>24</sup> was adopted with slight modification. The TSS were determined as °Brix at  $20 \pm 1^\circ\text{C}$  using a refractometer (Atago Master T, Tokyo, Japan). The device was lowered in each sample of carrot juice and reading was taken within 3 min. The test was performed in duplicate for each sample.

**Titrateable acidity:** Titrateable acidity of each sample of carrot juice was determined by neutralizing  $100 \text{ cm}^3$  of the carrot juice with 0.1 M of Sodium Hydroxide (NaOH) using phenolphthalein as indicator. The result was expressed as percentage of malic acid. The test was performed in duplicate for each sample.

**Sensory evaluation:** A total of 10 semi-trained panelists carried out a preference ranking test to assess the taste, aroma, mouthfeel, appearance, colour and overall acceptability of pasteurized and unpasteurized carrot juices stored at different temperatures. Ranking of the carrot juice in terms of colour was carried out by the panelist inside a cabinet where standard light was available. Carrot juice inside glass bottles were presented to the panelist in a similar manner commercialized juice is served. The preference ranking test was used by the panelist to assess the colour of the juice in a cabinet where there was a

standardized light. Twenty five milliliter of each sample of carrot juice was poured inside a labeled plastic glasses and served each panelist. The flavour of the samples were assessed independently by the panelist. Order of presentation of the carrot juices was based on a balanced-block design. Assessment of sensory attributes of each carrot juice involved the use of nine-point hedonic scale which range from 1 (dislike extremely) to 9 (like extremely)<sup>25</sup>.

**Statistical analysis:** Data obtained in replicates were subjected to One-way Analysis of Variance (ANOVA) with the help of IBM SPSS Statistics version 23 software. A probability value at  $p < 0.05$  was considered to be statistically significant. Duncan's Multiple Range Test (DMRT) was used to separate the means.

## RESULTS

The total heterotrophic bacterial count (THBC) of pasteurized and unpasteurized carrot juice samples stored at different temperatures (4 and 24°C) for 5 days was shown in Fig. 1. The THBC of unpasteurized carrot juice stored at 4°C and 24°C was within the range of 6.301 to 6.89 and 7.31 to 9.32  $\log_{10}$  CFU  $\text{mL}^{-1}$ , respectively. As for the pasteurized carrot juice stored at 4°C, the THBC was  $< 1.0 \log_{10}$  CFU  $\text{mL}^{-1}$  while the result for the samples stored at 24°C was within the range of 4.47 to 6.69  $\log_{10}$  CFU  $\text{mL}^{-1}$ . Figure 2 shows the THBC of pasteurized and unpasteurized carrot juice stored at 0°C for 5 weeks. At 0°C, the highest THBC recorded for unpasteurized and pasteurized carrot juice was 6.63 and 3.66  $\log_{10}$  CFU  $\text{mL}^{-1}$ , respectively.

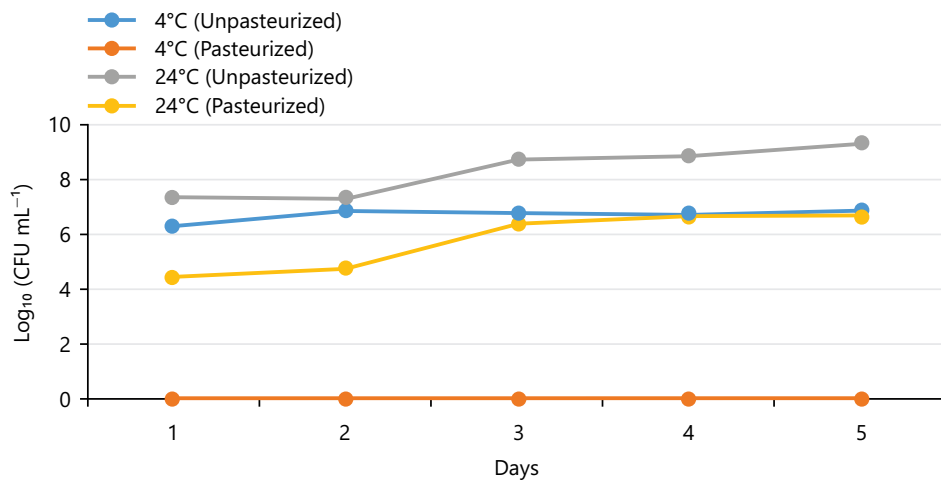


Fig. 1: Total heterotrophic bacterial count of pasteurized and unpasteurized carrot juices stored at different temperatures

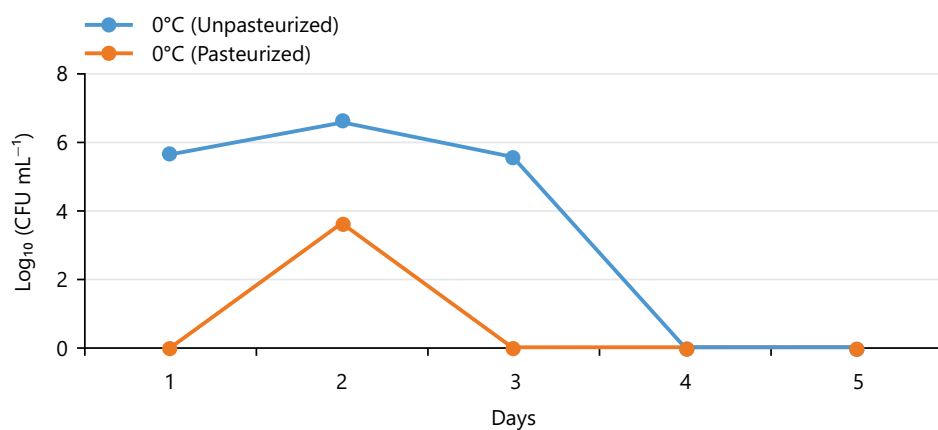


Fig. 2: Total heterotrophic bacterial count of pasteurized and unpasteurized carrot juices stored at 0°C for 5 weeks

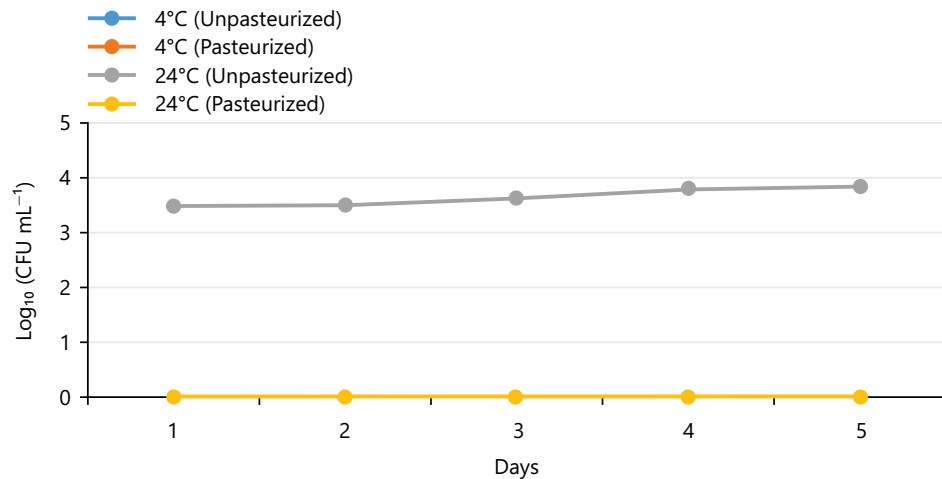


Fig. 3: Total fungal count of pasteurized and unpasteurized carrot juices stored at different temperatures for 5 days

Table 1: Gram stain, morphology and biochemical characteristics of bacterial isolates

Isolate code	Gram stain	Morphology	Catalase	Oxidase	Sucrose	Glucose	Lactose	Starch	TSIA slant	Agar butt	Gas	H <sub>2</sub> S	Indole	MR	VP	Citrate	Motility	Probable genera
A	+	R	+	-	+	+	-	-	B	A	-	+	-	+	-	+	+	<i>Bacillus</i> sp.
B	+	C	+	-	+	+	+	+	B	A	-	-	-	+	+	+	-	<i>Staphylococcus</i> sp.
C	-	R	+	+	+	+	-	+	B	B	-	-	-	+	-	+	+	<i>Pseudomonas</i> sp.
D	-	R	+	-	+	+	-	+	B	A	+	-	-	-	+	-	+	<i>Serratia</i> sp.

TSIA: Triple sugar iron agar, MR: Methyl red, VP: Voges proskauer, R: Rod, C: Cocci, A: Acid, B: Base, +: Positive and -: Negative

Figure 3 shows the total fungal count (TFC) of pasteurized and unpasteurized carrot juice stored at different temperatures (4 and 24°C) for 5 days. The TFC of unpasteurized carrot juice slightly increased from 3.5 to 3.85 log<sub>10</sub> CFU mL<sup>-1</sup> during the period of storage at 24°C whereas the result for pasteurized sample was <1.0 log<sub>10</sub> CFU mL<sup>-1</sup>. Within the same period of storage, the TFC of both pasteurized and unpasteurized carrot juice stored at 4°C was <1.0 log<sub>10</sub> CFU mL<sup>-1</sup>. A similar result was recorded for both pasteurized and unpasteurized carrot juice stored for 5 weeks at 0°C.

Table 1 shows the result of biochemical tests and Gram staining of bacterial isolates from both pasteurized and unpasteurized carrot juice. The result indicates that 4 bacterial genera which include *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Serratia* sp. were encountered in the carrot juice samples.

*Bacillus* sp. (43 %) which accounted for the highest frequency of occurrence was found in all the samples of unpasteurized and pasteurized carrot juice stored at 0, 4 and 24°C. It is followed by *Serratia* sp. (33%) reported in all carrot juice samples stored at different temperatures (0, 4 and 24°C). *Pseudomonas* sp. (16%) was isolated from unpasteurized carrot juice stored at 24°C and 4°C. The bacterial isolate which had the lowest frequency of occurrence was *Staphylococcus* sp. (8%) isolated from unpasteurized carrot juice stored at 24°C.

The fungi genera which had the highest frequency of occurrence was *Penicillium* spp. (45%) isolated from pasteurized and unpasteurized carrot juice stored at 24°C and 4°C for 5 days. It is followed by *Saccharomyces* spp. (37%) which was encountered in both pasteurized and unpasteurized carrot juice stored at different temperatures (4 and 24°C). *Aspergillus* spp. (18%) isolated from unpasteurized carrot juice stored at 24°C is the fungal genera that had the lowest frequency of occurrence.

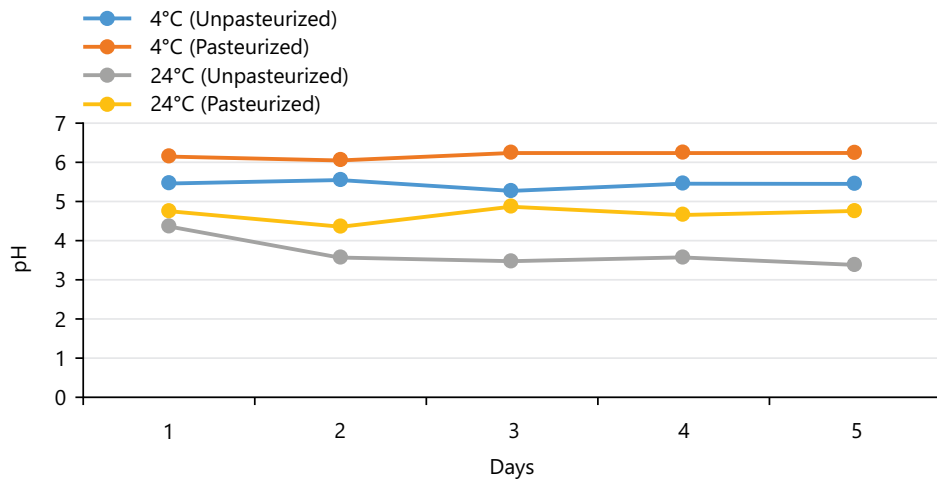


Fig. 4: pH of unpasteurized and pasteurized carrot juice stored at different temperatures

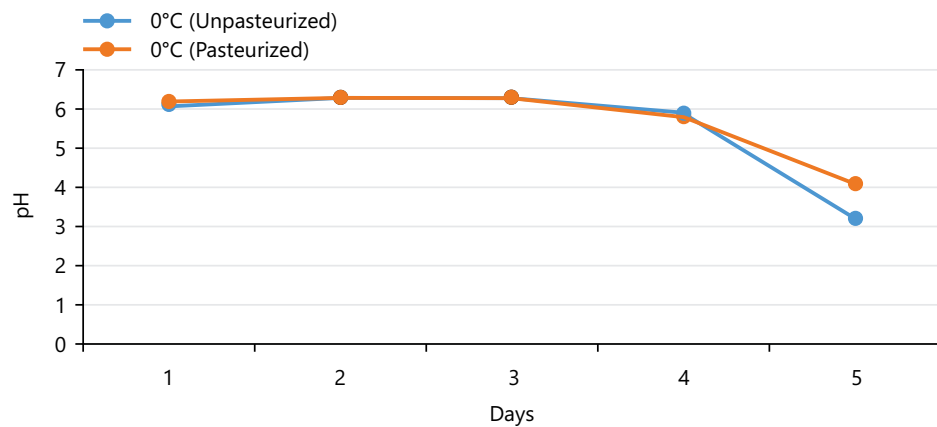


Fig. 5: pH of unpasteurized and pasteurized carrot juices stored for 5 days at 0°C

Figure 4 shows the pH of unpasteurized and pasteurized carrot juice stored at different temperatures (4 and 24°C) for 5 days. At 4°C, the pH of unpasteurized and pasteurized carrot drink was within the range of 5.3-5.6 and 6.1-6.3, respectively. When the storage temperature was increased to 24°C, the pH of unpasteurized and pasteurized carrot drink was within the range of 3.4-4.4 and 4.4-4.9, respectively. The pH of unpasteurized (3.2-6.3) and pasteurized (4.1-6.3) carrot juice stored for 5 weeks at 0°C was presented in Fig. 5.

Figure 6 shows the titratable acidity (TA) of pasteurized and unpasteurized carrot juice stored at 4 and 24°C for 5 days. The results shows there was a steady increase in TA of both samples with the exception of pasteurized carrot juice stored at 0°C which slightly reduced from 0.7 to 0.3% within 3 days of storage, but increased in subsequent days. Figure 7 shows the TA of pasteurized and unpasteurized carrot juice stored at 0°C for 5 weeks. Within 2 weeks of storage, there was a decrease in TA of both pasteurized and non-pasteurized carrot juice which later increased steadily in subsequent weeks.

Figure 8 shows the total soluble solids of pasteurized and unpasteurized carrot juice stored at different temperatures. During the period of storage, reduction in TSS in all the samples of carrot juice occurred except at few intervals when the values were the same or slightly increased. The TSS of unpasteurized carrot juice stored at 4 and 24°C was within the range of 14-19 and 8-23 °Brix. The TSS within the range of 7-16 and 8-11 °Brix was reported in pasteurized samples of carrot juice stored at 4 and 24°C, respectively for 5 days.

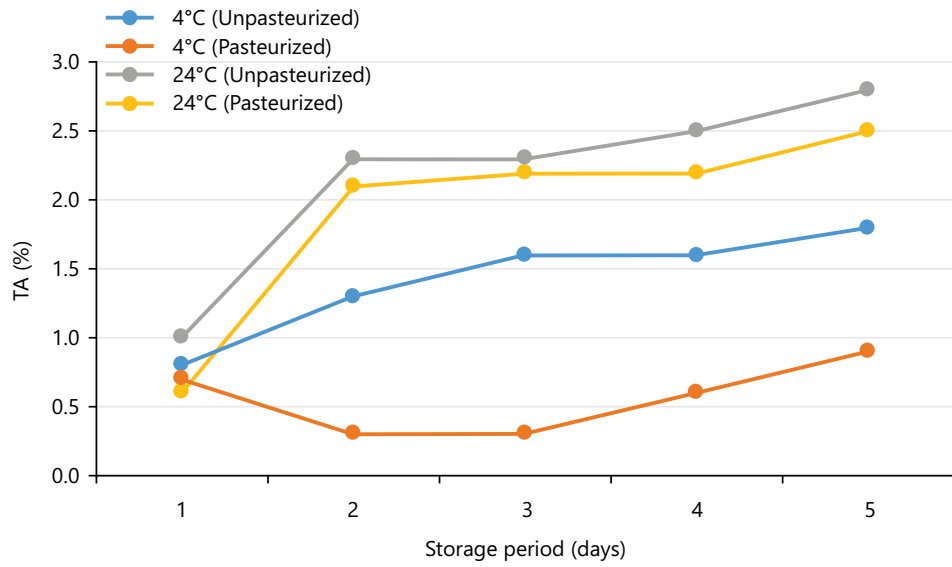


Fig. 6: Titratable acidity of pasteurized and unpasteurized carrot juices stored at different temperatures

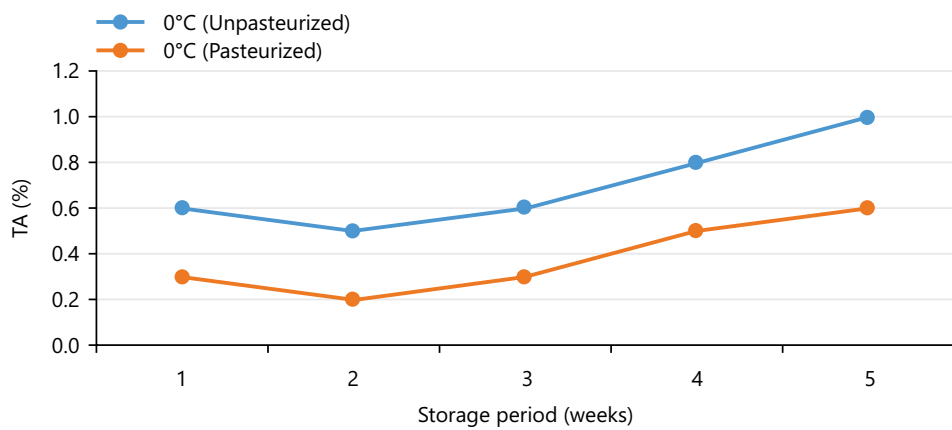


Fig. 7: Titratable acidity of pasteurized and unpasteurized carrot juices stored for 5 weeks at 0°C

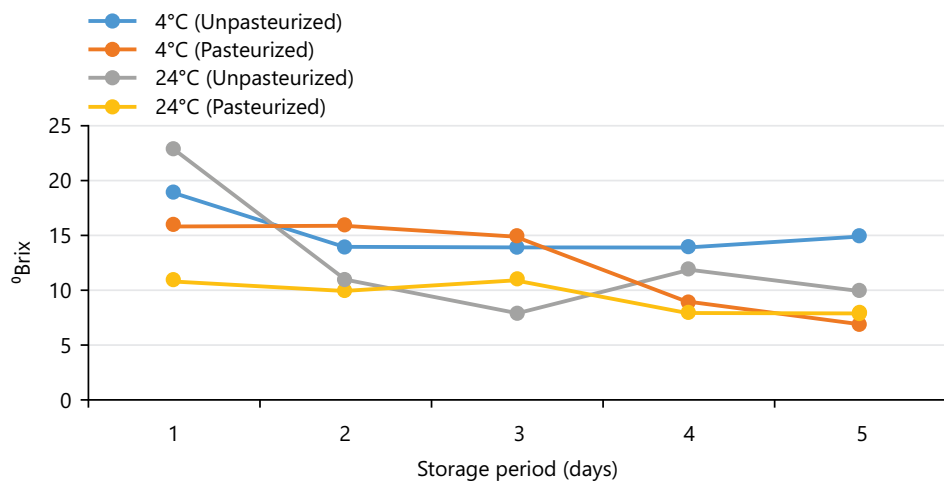


Fig. 8: Total soluble solids of pasteurized and unpasteurized carrot juices stored at different temperatures



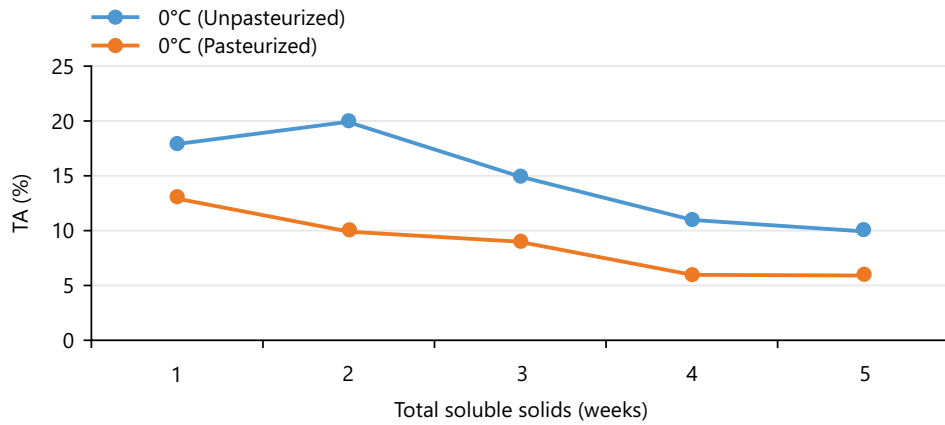


Fig. 9: Total soluble solids of pasteurized and unpasteurized carrot juices stored for 5 weeks at 0°C

Table 2: Sensory evaluation of pasteurized and unpasteurized carrot juices at day 1

Parameter	CAAP			CABP		
	CAAP1	CAAP2	CAAP3	CABP1	CABP2	CABP3
Taste	6.1±1.29 <sup>b</sup>	6.3±1.16 <sup>b</sup>	6.0±1.05 <sup>b</sup>	4.1±0.74 <sup>a</sup>	4.3±0.67 <sup>a</sup>	4.1±0.88 <sup>a</sup>
Aroma	6.2±0.63 <sup>b</sup>	5.4±1.07 <sup>b</sup>	5.4±1.58 <sup>b</sup>	3.4±0.70 <sup>a</sup>	3.1±1.10 <sup>a</sup>	4.0±1.49 <sup>a</sup>
Mouth feel	7.3±0.95 <sup>d</sup>	6.3±0.95 <sup>c</sup>	6.2±1.03 <sup>c</sup>	4.0±0.82 <sup>a</sup>	4.2±0.92 <sup>a</sup>	5.3±0.95 <sup>b</sup>
Appearance	8.0±1.05 <sup>b</sup>	7.2±0.92 <sup>ab</sup>	8.0±1.25 <sup>b</sup>	7.1±1.29 <sup>ab</sup>	8.1±1.10 <sup>b</sup>	6.2±1.23 <sup>a</sup>
Colour	8.1±0.88 <sup>ab</sup>	8.1±1.10 <sup>ab</sup>	7.1±1.20 <sup>a</sup>	9.0±0.00 <sup>b</sup>	8.0±1.25 <sup>a</sup>	7.2±1.14 <sup>a</sup>
Overall acceptability	7.1±0.99 <sup>c</sup>	7.1±1.10 <sup>c</sup>	7.3±0.95 <sup>c</sup>	5.4±0.84 <sup>b</sup>	4.4±0.84 <sup>a</sup>	5.0±1.05 <sup>ab</sup>

Values show means of sensory scores of ten panelists ±SD, Values with different superscript across the row are significantly different (p<0.05), Hedonic scale: 9: Like extremely, 8: Like very much, 7: Like moderately, 6: Like slightly, 5: Neither liked nor disliked, 4: Disliked slightly, 3: Disliked moderately, 2: Disliked very much, 1: Disliked extremely, CAAP1: Pasteurized carrot juice stored at 0°C, CAAP2: Pasteurized carrot juice stored at 4°C, CAAP3: Pasteurized carrot juice stored at 24°C, CABP1: Unpasteurized carrot juice stored at 0°C, CABP2: Unpasteurized carrot juice stored at 4°C, CABP3: Unpasteurized carrot juice stored at 24°C

Table 3: Sensory evaluation of pasteurized and unpasteurized carrot juices at day 5

Parameter	CAAP			CABP		
	CAAP1	CAAP2	CAAP3	CABP1	CABP2	CABP3
Taste	5.2±1.03 <sup>a</sup>	6.2±1.03 <sup>a</sup>	6.3±1.25 <sup>a</sup>	5.2±1.23 <sup>a</sup>	5.4±0.97 <sup>a</sup>	5.2±1.03 <sup>a</sup>
Aroma	5.1±0.99 <sup>a</sup>	5.4±1.17 <sup>a</sup>	5.3±0.82 <sup>a</sup>	5.0±0.94 <sup>a</sup>	5.4±0.84 <sup>a</sup>	5.1±0.99 <sup>a</sup>
Mouth feel	4.4±0.84 <sup>ab</sup>	4±0.82 <sup>a</sup>	5.3±0.95 <sup>b</sup>	5.1±1.20 <sup>ab</sup>	5.0±0.82 <sup>ab</sup>	5.1±1.20 <sup>ab</sup>
Appearance	6.1±0.99 <sup>abc</sup>	6.4±1.17 <sup>bc</sup>	6.0±1.25 <sup>ab</sup>	5.2±1.03 <sup>a</sup>	7.2±0.99 <sup>c</sup>	7.1±0.74 <sup>c</sup>
Colour	6.4±1.17 <sup>ab</sup>	7.2±1.03 <sup>b</sup>	7.0±0.94 <sup>ab</sup>	6.0±1.05 <sup>a</sup>	7.0±1.15 <sup>ab</sup>	7.0±0.94 <sup>ab</sup>
Overall acceptability	6.4±1.07 <sup>b</sup>	6.3±0.88 <sup>ab</sup>	6.3±1.34 <sup>b</sup>	5.1±0.99 <sup>a</sup>	6.0±1.05 <sup>ab</sup>	6.3±1.06 <sup>b</sup>

Values show means of sensory scores of ten panelists ±SD, Values with different superscript across the row are significantly different (p<0.05), hedonic scale: 9: Like extremely, 8: Like very much, 7: Like moderately, 6: Like slightly, 5: Neither liked nor disliked, 4: Disliked slightly, 3: Disliked moderately, 2: Disliked very much, 1: Disliked extremely, CAAP1: Pasteurized carrot juice stored at 0°C, CAAP2: Pasteurized carrot juice stored at 4°C, CAAP3: Pasteurized carrot juice stored at 24°C, CABP1: Unpasteurized carrot juice stored at 0°C, CABP2: Unpasteurized carrot juice stored at 4°C and CABP3: Unpasteurized carrot juice stored at 24°C

Figure 9 shows the total soluble solids (TSS) of pasteurized and unpasteurized carrot juices stored at 0°C for 5 weeks. In both samples of carrot juice, there was a steady reduction in TSS during the period of storage with the exception of unpasteurized carrot juice which slightly increased at week 2. The TSS of unpasteurized and pasteurized carrot juice stored at 0°C was within the range of 10-20 and 6-13 °Brix, respectively.

The sensory evaluation report of pasteurized and unpasteurized carrot juice at day 1 is presented in Table 2. According to the report, pasteurized carrot juice stored at 0, 4 and 24°C had a higher sensory scores compared to unpasteurized juices stored under the same condition. Table 3 shows the sensory

evaluation report of pasteurized and unpasteurized carrot juices stored at different temperatures at day 5. The report shows that pasteurized carrot juices had a slightly higher scores for all the sensory attributes compared to unpasteurized carrot juices with few except.

## DISCUSSION

The result obtained from this study shows that the total heterotrophic bacterial count (THBC) of unpasteurized carrot juice was higher than that pasteurized juice. It could be attributed to high temperature involved in pasteurization which killed a lot of microbes present in carrot juice. This report was in agreement with the findings of Jimma *et al.*<sup>26</sup> In addition, low temperature storage could also have contributed to reducing the microbial load of the carrot juices. Kaddumukasa *et al.*<sup>27</sup>, reported a similar trend in a related study. According to Oranusi *et al.*<sup>28</sup>, edible products containing microbial contaminants below  $6 \log_{10}$  CFU mL<sup>-1</sup> are acceptable for humans to consume. Meanwhile, the International Commission on Microbiological Specifications of Food (ICMSF) and the National Agency for Food and Drug Administration and Control (NAFDAC) stipulate that mesophilic aerobic bacterial count in fruit juice should not be above  $3 \log_{10}$  CFU mL<sup>-1</sup><sup>29</sup>. Codex Alimentarius Commission (CAC) of the Food and Agricultural Organization (FAO) recommend that the total mean plate count of fruit juice should not exceed  $1 \times 10^5$  CFU mL<sup>-1</sup><sup>27</sup>. Pasteurized carrot juice stored at 4°C for 5 days met the ICMSF and NAFDAC standards. A similar result also involved carrot juices stored at 0°C for 5 weeks except that THBC of the pasteurized juice exceeded the recommended limit at week 2; the unpasteurized carrot juice at weeks 1, 2 and 3. Pasteurized and unpasteurized carrot juices stored at 24°C for 5 days exceeded the permissible limit for mesophilic aerobic bacterial count recommended by the CAC, ICMSF and NAFDAC.

Asghar *et al.*<sup>30</sup> reported that total plate count, *Staphylococcus aureus*, yeast and mould count of fresh carrot juice sold by vendors is within the range of  $4.5 \times 10^3$ - $5.5 \times 10^5$ ,  $<10$ - $6.0 \times 10^3$  and  $6.7 \times 10^1$ - $8.5 \times 10^3$  per millilitre of the juice, respectively. Total coliforms, fecal coliform and *Escherichia coli* were not detected in some samples of the juice, but the highest values encountered in others were 460, 240 and 93 MPN mL<sup>-1</sup>, respectively. *Salmonella* sp., was detected in 3 out of 10 samples of the carrot juice. In a related study, Asha *et al.*<sup>31</sup> reported that the TVC and coliform count of carrot juice sold to the public is within the range of  $80$ - $271 \times 10^5$  CFU mL<sup>-1</sup> and  $1$ - $54 \times 10^5$  CFU mL<sup>-1</sup>, respectively. Both reports are in agreement with the results obtained from this study. Ghosh *et al.*<sup>32</sup> reported that total aerobic count, total staphylococcal count, total coliforms, total fecal coliforms of carrots obtained from street vendors before washing the root vegetable is within the range of 5.0-7.98, 4.81-7.56, 2.0-5.97 and 3.2-8.2  $\log_{10}$  CFU mL<sup>-1</sup> while the values reported for washed carrots is 3.1-6.4, 1.25-5.0, 2.08-6.67 and 5.80-8.33  $\log_{10}$  CFU g<sup>-1</sup>, respectively. Fruits and vegetables are easily contaminated by microorganisms when they are allowed to fall on the ground during harvesting. The use of dirty water to wash fruits and vegetables could contaminate them with fecal pathogens. Other possible sources of microbial contamination of carrot juice include dust, air and personnel during the preparation of the juice<sup>27</sup>.

Our results showed that unpasteurized carrot juice stored at 24°C for 5 days had a total fungal count within the range of 3.5-3.85  $\log_{10}$  CFU mL<sup>-1</sup>. Other samples of carrot juice stored at 4°C and 24°C were  $<1.0 \log_{10}$  CFU mL<sup>-1</sup>. The effect of pasteurization and low-temperature storage could be responsible for the low TFC of the carrot juices. Poor storage conditions and improper handling of carrots beginning from the stage of harvesting to processing of carrots into juice are possible sources of fungal contamination of the ready-to-drink juice<sup>33</sup>. Asha *et al.*<sup>31</sup> reported that the yeast count of street vended carrot juice is within the range of  $5$ - $41 \times 10^5$  CFU mL<sup>-1</sup>.

The dominance of *Bacillus* sp. in the carrot juice samples stored at different temperatures could be attributed to the bacterium being ubiquitous in nature and a spore former<sup>19</sup>. The presence of bacterial species in the carrot juice is an indication they can survive in an acidic environment. This is probably because the organisms are capable of regulating their internal pH which they adjusted to neutral by

deploying two mechanisms-passive and active homeostasis<sup>34</sup>. According to Oranusi *et al.*<sup>28</sup>, bacterial species encountered in an edible product shows the quality of raw materials, the hygienic status of the environment, process equipment, packaging materials and personnel handling the product.

*Serratia marscescens* was reported in carrots sold in selected markets in Ekiti State<sup>18</sup>. *Serratia* sp., is a bacterium whose presence in processing plants, water, soil, insects and vertebrates has been reported. Aneja *et al.*<sup>34</sup> reported that *Serratia* sp., contaminated freshly prepared carrot juice. This is in agreement with the findings from this study. According to Adeleke *et al.*<sup>18</sup>, *Serratia marscescens* is associated with meningitis in children.

With the exception of *Pseudomonas* sp., all the bacterial genera encountered in carrot juices were reported in a related study by Aneja *et al.*<sup>34</sup>. *Pseudomonas* sp., is a common food contaminant associated with spoilage. Morka<sup>35</sup> reported that *Pseudomonas aeruginosa* is among the bacteria implicated in the spoilage of carrots sold in the market. According to Igiehon *et al.*<sup>36</sup> *Pseudomonas* sp., isolated from vended fruits could cause necrotizing inflammation in the body.

*Staphylococcus* sp., isolated from the carrot juice could be the specie that usually inhabit the skin, hair, palm, nose and mucus membrane as part of the normal flora. The bacterium is commonly associated with post-process contamination of food products such as carrot juice<sup>28</sup>. A possible source of contamination of carrot juice by *Staphylococcus* sp., is the food handlers. According to Ire *et al.*<sup>37</sup> carbuncles, furuncles, folliculitis, erysipelas, scalded skin syndrome (toxemia) and cellulitis are human diseases associated with *Staphylococcus aureus*.

According to Oranusi *et al.*<sup>28</sup> spoilage of fruits and vegetables is usually caused by fungi such as *Aspergillus* and *Penicillium* species. Aneja *et al.*<sup>34</sup> reported that freshly prepared carrot juice was contaminated by *Saccharomyces* sp., *Aspergillus flavus*, *A. terreus*, *A.niger* and *Penicillium islandicum*. The study further revealed that *Aspergillus flavus* and *Saccharomyces* sp., were among the dominant mould and yeast encountered in the carrot juice, respectively. The result is reasonably in agreement with the findings from this study.

There was a slight reduction in the pH of unpasteurized carrot juice stored at 24°C for 5 days from 4.4-3.4. This could be attributed to favourable temperature for fermentative bacteria in the carrot juice to carry out their activities. Secondly, an increase in titratable acidity in the samples could also be a contributory factor because of the inversely proportional relationship between pH and titratable acidity. Hashem *et al.*<sup>38</sup> reported that pH of freshly prepared carrot juice is  $6.35 \pm 0.16$ . The standard for pH of fruit juice recommended by FAO/WHO and Standard Organization of Nigeria (SON) is 1.4-4.0<sup>39</sup>.

The titratable acidity (TA) of pasteurized and unpasteurized carrot juice stored at different temperatures increased during the period of storage. Increase in acidity of pasteurized carrot juice stored at room temperature from  $0.05 \pm 0.01$ - $0.08 \pm 0.01\%$  reported by Kapoor and Aggarwal<sup>40</sup> is in agreement with the trend reported in this study. Hashem *et al.*<sup>38</sup> reported that acidity of freshly prepared carrot juice is  $0.38 \pm 0.17\%$ . The increase in TA of carrot juice during the period of storage could be attributed to organic acid undergoing partial hydrolysis<sup>41</sup>.

The result from this study showed that during storage of carrot juice at different temperatures, there was a reduction in total soluble solids (TSS). It could be attributed to the activities of microorganisms that break down sugar in the juices and become acids<sup>42</sup>. In a related study, Kapoor and Aggarwal<sup>40</sup> reported that TSS of tightly corked and pasteurized carrot juice stored at room temperature for 6 months reduced from  $10 \pm 0.03$ - $9.73 \pm 0.11$  °Brix. This is in agreement with our results. Hashem *et al.*<sup>38</sup> reported that the total soluble solids in freshly prepared carrot juice are  $4.17 \pm 0.01$  °Brix.

The sensory evaluation report of freshly prepared carrot juices on day 1 showed that there was no significant difference ( $p>0.05$ ) in taste and aroma among the pasteurized and unpasteurized samples stored at 0, 4 and 24°C. Similarly, there was no significant difference ( $p>0.05$ ) in overall acceptability among the pasteurized samples which contradicted the report of unpasteurized samples. This result could be attributed to pasteurization of carrot juice which significantly reduced the population of microorganisms capable of releasing metabolites into the juice that will influence its overall acceptability. The sensory report of day 1 indicates that pasteurized carrot juice stored at 0, 4 and 24°C was preferable to the samples which were unpasteurized. On day 5, the taste and aroma among the carrot juice samples were not significantly different ( $p>0.05$ ). It is worth noting that sensory scores for mouthfeel, appearance, colour and overall acceptability of pasteurized and unpasteurized carrot juice samples on day 1 were higher than what was reported in the samples on day 5. This could be attributed to the activities of microorganisms in the products which increased in population during the period of storage. Consequently, the activities of the microorganisms probably were responsible for breaking down sugar in carrot juice and releasing metabolites which impacted the sensory properties of the product.

## **CONCLUSION**

This study has shown that unpasteurization of carrot juice and storage of the product at room temperature which are common practices in many households make the product unfit for human consumption. However, pasteurization and storage of carrot juices at low temperatures improved the microbiological and sensorial quality of the product. Further studies on the effect of pasteurization and storage temperatures on the proximate composition and mineral content of homemade carrot juice is recommended.

## **SIGNIFICANCE STATEMENT**

Increasing concern about the health effects of artificial flavours and preservatives in many brands of fruit and vegetable juices has made homemade juice to be a good alternative. Unpasteurization and room temperature storage associated with homemade juice could affect its quality and pose a health risk to consumers. The effect of both conditions on the microbiological, physicochemical and sensory parameters of carrot juice was determined. A bacterial load of pasteurized carrot juice stored at 4°C for 5 days; at 0°C for 5 weeks was within the recommended limits while changes in pH, titratable acidity and total soluble solids occurred. The pasteurized carrot juice stored at different temperatures had better sensory attributes compared with the unpasteurized juices.

## **ACKNOWLEDGMENTS**

The authors hereby appreciate the support given to us by the Chief Laboratory Technologist and other support staff at General Microbiology Laboratory, University of Port Harcourt, during the laboratory work.

## **REFERENCES**

1. Potter, A.S., S. Foroudi, A. Stamatikos, B.S. Patil and F. Deyhim, 2011. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutr. J.*, Vol. 10. 10.1186/1475-2891-10-96.
2. Raggio, L. and A. Gámbaro, 2018. Study of the reasons for the consumption of each type of vegetable within a population of school-aged children. *BMC Public Health*, Vol. 18. 10.1186/s12889-018-6067-4.
3. Pem, D. and R. Jeewon, 2015. Fruit and vegetable intake: Benefits and progress of nutrition education interventions- Narrative review article. *Iran. J. Public Health*, 44: 1309-1321.
4. Ajenu, C.O., C. Imoisi, E.E. Imhontu and U.R.Orji, 2021. Comparative evaluation of the proximate and micro-nutritional benefits of pawpaw, carrot, turmeric and coconut. *J. Food Technol. Nutr. Sci.*, 3: 1-5.
5. Yahia, E.M., P. García-Solís and M.E.M. Celis, 2019. Contribution of Fruits and Vegetables to Human Nutrition and Health. In: *Postharvest Physiology and Biochemistry of Fruits and Vegetables*, Yahia, E.M. (Ed.), Woodhead Publishing, Sawston, Cambridge, ISBN: 9780128132784, pp: 19-45.

6. Surbhi, S., R.C. Verma, R. Deepak, H.K. Jain and K.K. Yadav, 2018. A review: Food, chemical composition and utilization of carrot (*Daucus carota* L.) pomace. *Int. J. Chem. Stud.*, 6: 2921-2926.
7. Que, F., X.L. Hou, G.L. Wang, Z.S. Xu and G.F. Tan *et al.*, 2019. Advances in research on the carrot, an important root vegetable in the Apiaceae family. *Hortic. Res.*, Vol. 6. 10.1038/s41438-019-0150-6.
8. Boadi, N.O., M. Badu, N.K. Kortei, S.A. Saah and B. Annor *et al.*, 2021. Nutritional composition and antioxidant properties of three varieties of carrot (*Daucus carota*). *Sci. Afr.*, Vol. 12. 10.1016/j.sciaf.2021.e00801.
9. Varshney, K. and K. Mishra, 2022. An analysis of health benefits of carrot. *Int. J. Innovative Res. Eng. Manage.*, 9: 211-214.
10. da Silva Dias, J.C., 2014. Nutritional and health benefits of carrots and their seed extracts. *Food Nutr. Sci.*, 5: 2147-2156.
11. Bratte, A.G., A.S. Adeleye and A.S. Oyerinde, 2016. Comparison of nutritional and colour properties of fresh and dried carrot (*Daucus carota* L.) slices and carrot pomace. *J. Multidiscip. Eng. Sci. Technol.*, 3: 5366-5370.
12. Aderinola, T.A. and K.E. Abaire, 2019. Quality acceptability, nutritional composition and antioxidant properties of carrot-cucumber juice. *Beverages*, Vol. 5. 10.3390/beverages5010015.
13. Prasad, S., 2021. Development, analysis and sensory evaluation of carrot squash as a natural health drink. *Int. J. Innovative Res. Technol.*, 8: 1071-1077.
14. Ahmad, T., M. Cawood, Q. Iqbal, A. Ariño and A. Batool *et al.*, 2019. Phytochemicals in *Daucus carota* and their health benefits-review article. *Foods*, Vol. 8. 10.3390/foods8090424.
15. Hager, T.J. and L.R. Howard, 2006. Processing effects on carrot phytonutrients. *Am. Soc. Hortic. Sci.*, 41: 74-79.
16. Singh, M.N., R. Srivastava and I. Yadav, 2021. Study of different varieties of carrot and its benefits for human health: A review. *J. Pharmacogn. Phytochem.*, 10: 1293-1299.
17. Hammad, A.A., H.H. Abd El-Khalek, K.A. Youssef and R.M. Abd El-Kader, 2013. Microbiological, nutritional and sensorial changes in fresh carrot juice preserved by irradiation. *Food Sci. Qual. Manage.*, 11: 61-69.
18. Adeleke, M.A., A.O. Hassan, T.T. Ayepola, T.M. Famodimu, W.O. Adebimpe and G.O. Olatunde, 2012. Public health risks associated with apples and carrots sold in major markets in Osogbo, Southwest Nigeria. *J. Toxicol. Environ. Health Sci.*, 4: 140-144.
19. Ahaotu, I., M.O. Ichendu and N. Maduka, 2022. Microbiological, nutritional and sensory evaluation of snack bars developed using Bambara groundnut (*Vigna subterranean* L.) and maize (*Zea mays*). *Afr. J. Microbiol. Res.*, 16: 8-23.
20. Bergey, D.H. and J.G. Holt, 1994. *Bergey's Manual of Determinative Bacteriology*. 9th Edn., Williams and Wilkins, Baltimore, Maryland, ISBN: 978068300603, Pages: 787.
21. Shoaib, M., I. Muzammil, M. Hammad, Z.A. Bhutta and I. Yaseen, 2020. A mini-review on commonly used biochemical tests for identification of bacteria. *Int. J. Res. Publ.*, Vol. 54. 10.47119/IJRP100541620201224.
22. Palma, V., M.S. Gutiérrez, O. Vargas, R. Parthasarathy and P. Navarrete, 2022. Methods to evaluate bacterial motility and its role in bacterial-host interactions. *Microorganisms*, Vol. 10. 10.3390/microorganisms10030563.
23. Kidd, S., C.L. Halliday, H. Alexiou and D.H. Ellis, 2016. *Descriptions of Medical Fungi*. 3rd Edn., CutCut Digital, New York, ISBN-13: 9780646951294, Pages: 264.
24. Kong, T.Y., N.Z.N. Hasnan, A.N. Diyana, I.M. Nurin-Zulkifli, R.K. Basha, N.H. Abdul Ghani and N.A. Aziz, 2020. Effect of mild-temperature and high-temperature pasteurisation on physicochemical properties, bioactive compound, antioxidant activity and microbiological qualities of reconstituted pomegranate juice (RPJ). *Food Res.*, 4: 157-164.
25. Tiencheu, B., D.N. Nji, A.U. Achidi, A.C. Egbe and N. Tenyang *et al.*, 2021. Nutritional, sensory, physico-chemical, phytochemical, microbiological and shelf-life studies of natural fruit juice formulated from orange (*Citrus sinensis*), lemon (*Citrus limon*), Honey and Ginger (*Zingiber officinale*). *Heliyon*, Vol. 7. 10.1016/j.heliyon.2021.e07177.

26. Jimma, F.I., A. Mohammed, E.G. Adzaworlu, J. Nzeh, L. Quansah and O.A. Dufailu, 2022. Microbial quality and antimicrobial residue of local and industrial processed fruit juice sold in Tamale, Ghana. Discover Food, Vol. 2. 10.1007/s44187-022-00028-2.
27. Kaddumukasa, P.P., S.M. Imathiu, J.M. Mathara and J.L. Nakavuma, 2017. Influence of physicochemical parameters on storage stability: Microbiological quality of fresh unpasteurized fruit juices. Food Sci. Nutr., 5: 1098-1105.
28. Oranusi, S.U., W. Braide and H.O. Neziyanya, 2012. Microbiological and chemical quality assessment of some commercially packed fruit juices sold in Nigeria. Greener J. Biol. Sci., 2: 1-6.
29. Obasi, B.C., C.M.Z. Whong, J.B. Ameh and E.E. Ella, 2019. Microbiological quality assessment of commercially and laboratory prepared orange juice. J. Biotechnol. Res., 5: 19-27.
30. Asghar, U., M. Nadeem, R. Nelofer, S. Mazhar, Q. Syed and M. Irfan, 2018. Microbiological assessment of fresh juices vended in different areas of Lahore City. Electron. J. Biol., 14: 106-110.
31. Asha, S., K. Nithisha, G. Niteesha, K.R. Bharath and V. Ravikumar, 2014. Evaluation of microbial quality of street vended vegetable and fruit juices. Int. Res. J. Biol. Sci., 3: 60-64.
32. Ghosh, M., A. Ganguli and S. Mudgil, 2004. Microbiological quality of carrots used for preparation of fresh squeezed street vended carrot juices in India. J. Food Agric. Environ., 2: 143-145.
33. Batool, S.A., S.S. Tahir, N. Rauf and R. Kalsoom, 2013. Microbiological analysis of pasteurized and fresh fruit juice sold in Rawalpindi of Pakistan. Bangladesh J. Sci. Ind. Res., 48: 185-192.
34. Aneja, K.R., R. Dhiman, N. K. Aggarwal, V. Kumar and M. Kaur, 2014. Microbes associated with freshly prepared juices of citrus and carrots. Int. J. Food Sci., Vol. 2014. 10.1155/2014/408085.
35. Morka, E., 2022. Isolation of some pathogenic microbes associated with spoiled carrots (*Daucus carota* L.) obtained from local markets in Abraka, Delta State, Nigeria. Dutse J. Pure Appl. Sci., 8: 1-8.
36. Igiehon O.O., A.E. Adekoya and A.T. Idowu, 2020. A review on the consumption of vended fruits: microbial assessment, risk, and its control. Food Quality and Safety 4: 77-81.
37. Ire, F.S., G.K. Benneth and N. Maduka, 2020. Microbiological evaluation of ready-to-drink tigernut drinks sold within port harcourt metropolis, rivers state, Nigeria. Asian Food Sci. J., 16: 45-58.
38. Hashem, H.A., A.M. Sharaf, S.A. Amira and G.E. Ibrahim, 2014. Changes in physico-chemical quality and volatile compounds of orange-carrot juice blends during storage. Food Sci. Qual. Manage., 33: 21-35.
39. Okoye, C.O.B. and C.N. Ibetu, 2009. Analysis of different brands of fruit juice with emphasis on their sugar and trace metal content. Bio-Research, 7: 493-495.
40. Kapoor, S. and P. Aggarwal, 2014. Effect of processing and storage on bioactive compounds and antioxidant activity of carrot juice. J. Appl. Hortic., 16: 80-84.
41. Babarinde, G.O., S.J. Olatunde and A. Adebisi-Olabode, 2019. Quality attributes and phytochemical properties of fresh juice produced from selected mango varieties. Ceylon J. Sci., 48: 31-36.
42. Mathew, B., V. Mini, J.M. Kuriakose, V.R. Shajan and G. Jayakumar, 2016. Effect of thermo chemical processing on storability of sugarcane juice. Int. J. Environ. Agric. Biotechnol., 1: 526-530.