



Research Article

Preservative Potentials of June Plum Extract on Watermelon Juice (*Citrullus* spp.)

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ABSTRACT

Background and Objective: Microorganisms spoil food and beverages, reducing quality and posing health risks. To extend shelf life, different methods like refrigeration, pasteurization and preservatives are used. This research aimed to evaluate the potential of June plum derived from citric acid as a natural preservative for extending the shelf life of watermelon juice. **Materials and Methods:** The experiments were designed using a Box-Behnken experimental design within the framework of response surface methodology. The measured parameters were total viable count, total coliform count, total fungi count, pH and titratable acidity over a 28-day storage period. The watermelon juice was prepared and pasteurized, while citric acid was extracted from June plum. The Independent variables were citric acid concentration (0.5-10 g), pasteurization time (10-20 sec) and storage temperature (0-30 °C). **Results:** Total viable count, total coliform count, total fungi count over 28 days ranged from 2.60 (Day 7) -4.45 (day 28) CFU/mL, 0 (day 7) -378 (day 28) CFU/mL, 0.02 (day 7) -1.17 (day 28) CFU/mL; pH over 28 days ranged from 4.10 (day 0) -3.53 (day 28). The titratable acidity ranged from 0.82-2.79 over 28 days. The study established that the total viable count, total coliform count and total fungi count remained within acceptable limits for up to 28 days when the fruit juice was preserved with citric acid and stored at 0, 15 and 30 °C. **Conclusion:** The study showed that the application of June plum derived from citric acid, along with controlled pasteurization time and storage temperature, effectively inhibited microbial growth and maintained the quality of watermelon juice over a 28-day period.

Key words: June plum, watermelon juice, citric acid, fruit juice, natural preservatives, microbial quality, food preservation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The spoilage of food and beverages due to the growth of microorganisms poses a risk to human health and decreases the quality of these products. To address this issue, various preservation methods have been employed, including the use of preservatives such as citric acid, which is effective against a broad range of microorganisms¹. Citric acid is commonly used in the food industry as a preservative, flavoring agent and acidifier and it has been found to extend the shelf life of many foods and beverage products². Additionally, citric acid enhances the flavor and texture of food and beverage products and has been found to be more effective than other organic acids in inhibiting the growth of microorganisms². *Spondias dulcis*, commonly known as June plum, is a tropical fruit tree rich in vitamins C and A, potassium and antioxidants. This fruit has demonstrated antimicrobial, antidiabetic and anti-inflammatory properties³, suggesting potential therapeutic uses. Similarly, watermelon, which is rich in bioactive compounds and antioxidants, has gained popularity due to its sensorial, physical and nutritional characteristics⁴. Freshly squeezed watermelon juice boasts immense nutritional value and refreshing taste but its inadequate shelf life poses a significant challenge. This short shelf life is primarily due to enzymatic browning, microbial spoilage and vitamin degradation, leading to rapid quality deterioration and food waste. This not only reduces the economic viability of the product but also hinders consumers' ability to enjoy the full benefits of fresh watermelon juice. The underutilized potential of June plum in addressing these challenges is noteworthy. Based on the literature review, it appeared that there was limited research on the potential use of citric acid extracted from June plum as a preservative in watermelon juice. Although studies have been conducted on the use of citric acid as a preservative in other fruit juices, the potential of June plum citric acid as a preservative in watermelon juice remains largely unexplored. Therefore, this study investigated the preservative potential of citric acid extracted from June plum in watermelon juice.

MATERIALS AND METHODS

Study area: The study was carried out at Nnamdi Azikiwe University Awka, Nigeria from May 2022 to April 2024.

Sources of raw material: The raw materials were sourced from various locations. June plum was procured from the Mile 1 market in Port Harcourt, Rivers State, while watermelon was

obtained from Eke Awka Market. Both the watermelon juice and June plum juice were extracted in the Food Science and Technology Laboratory at Nnamdi Azikiwe University in Awka.

Sample preparation

Production of June plum juice (Unripe): The June Plum juice was made using a slightly modified version of the procedure outlined by Cendrès *et al.*⁴. A complete bag (25 kg) of unripe June plums was cleaned and peeled, the seed was removed and the meat diced after the spoiled ones were sorted out. Juice was extracted using a Kenwood juicer (model: HHB 100E, Ajanta Limited, Morbi, India). After that, the juice was corked, preserved for future research and filtered into a container.

Production of watermelon juice: June Plum Juice was produced following the methodology outlined by Kumar *et al.*⁵. Before extraction of the juice, the whole watermelon was thoroughly washed to remove surface contaminants. After washing, the watermelon was cut open and diced into smaller pieces to facilitate processing. These diced pieces were then fed into a juicer, which extracted the juice while separating the pulp and seeds. This process yielded 4500 mL of watermelon juice. The fresh juice was then pasteurized at a constant temperature of 75°C for 10-20 sec, depending on the sample, to eliminate harmful bacteria and enzymes, thereby extending the shelf life of the juices. Following pasteurization, the juice was rapidly cooled to a safe temperature to preserve its flavor and quality while preventing further bacterial growth.

Extraction of citric acid crystals from June plum juice: The analytical grade sodium hydroxide, calcium chloride and sulfuric acid were used in the experiments without any further purification. The experimental procedures involved a three-step chemical synthesis of citric acid from fruit juices: (i) pH adjustment to 10 using a 2.8 M NaOH solution, (ii) addition of a CaCl₂ solution (40.3 - 41.1% w/v) and (iii) acidification with a H₂SO₄ solution (1.5-2.3 M) to produce citric acid. During neutralization, the 2.8 M NaOH solution (10% w/w) was gradually added to the June plum juices to achieve a pH of 10, allowing sodium citrate to remain soluble while other products were precipitated. After removing the insoluble components, the filtrate, containing an aqueous solution of sodium citrate, was filtered three times before proceeding to the next step.

In the second step, 500 mL of a 40.7% (w/v) CaCl₂ solution was added to the sodium citrate solution, which was then heated in a boiling water bath for 30 min, resulting in the

precipitation of calcium citrate. The mixture containing calcium citrate was vacuum-filtered and the residue was washed with 100 mL of hot water in four steps to remove impurities and byproducts. The filtrate was maintained at a neutral pH of 7 and the residue was dried to a constant weight in a hot air oven. The dried calcium citrate was then acidified with 250 mL of dilute H_2SO_4 (1.9 M) at 60 °C while being stirred with a glass rod. This process caused the separation of calcium citrate and calcium sulfate, both insoluble in water, with calcium sulfate settling at the bottom and the citric acid solution remaining on top. The mixture was vacuum-filtered similarly to the second step. Finally, citric acid was crystallized from its aqueous solution through evaporative crystallization, yielding 293 g of citric acid crystals, which were characterized using FTIR and gravimetrically quantified⁶:

$$\text{Yield of citric acid (\%)} = \frac{\text{Mass of citric acid in product}}{\text{Volume of juice used}} \times 100 \quad (1)$$

Experimental design: The face-centred central composite design (FCCD) was used in this research using Design Expert software version 13. Table 1 shows the process variables and their levels. The experimental matrix that was used in this study, based on a central composite face-centered design is shown in Table 2. The experimental space had a total of twenty (20) samples. Sample 21 (Ctrl⁻) is the Watermelon juice with no citric acid while sample 22 (Ctrl⁺) is the watermelon

juice with commercially made citric acid. The data obtained from the study was fitted to the second-order polynomial regression model of the form:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 + b_{12}AB + b_{13}AC + b_{23}BC + e \quad (2)$$

Where

- Y : Response Parameters
- b_0 : intercept
- b_1 - b_{23} : Coefficient estimate of the linear, interaction and square terms
- A : Citric Acid Concentration (mL)
- B : Pasteurization time (sec)
- C : Storage Temperature (°C)
- e : Estimated Error

Methods of analysis

pH measurement: The pH was determined by the use of a pH meter (pHS-3C), it was manufactured by OMEGA Engineering

Table 1: Key depicting independent variables and their levels of replication

Parameters	Levels of factors		
	-1	0	+1
Citric acid Conc (g) A	0.5	5.25	10
Pasteurization time (s) B	10	15	20
Storage temp (°C) C	0	15	30

Table 2: Central Composite face-center (CCFC) design matrix and the independent variables and their actual levels and coded values

Runs	Factor 1	Factor 2	Factor 3
	A: Citric acid conc. (g)	B: Pasteurization time (sec)	C: Storage Temp (°C)
1	10 (+1)	20 (+1)	0 (-1)
2	10 (+1)	10 (-1)	0 (-1)
3	5.25 (0)	15 (0)	15 (0)
4	5.25 (0)	15 (0)	15 (0)
5	5.25 (0)	15 (0)	15 (0)
6	5.25 (0)	15 (0)	30 (+1)
7	5.25 (0)	15 (0)	15 (0)
8	0.5 (-1)	20 (+1)	30 (+1)
9	5.25 (0)	10 (-1)	15 (0)
10	5.25 (0)	15 (0)	15 (0)
11	0.5 (-1)	20 (+1)	0 (-1)
12	0.5 (-1)	10 (-1)	0 (-1)
13	10 (+1)	20 (+1)	30 (+1)
14	0.5 (-1)	15 (0)	15 (0)
15	5.25 (0)	15 (0)	15 (0)
16	5.25 (0)	20 (+1)	15 (0)
17	5.25 (0)	15 (0)	0 (-1)
18	10 (+1)	15 (0)	15 (0)
19	10 (+1)	10 (-1)	30 (+1)
20	0.5 (-5)	10 (-1)	30 (+1)
CTRL ⁺	2.5	20	0
CTRL ⁻	0	20	0

Values in bracket are the coded values while the ones not in bracket are the actual values

Inc located in Norwalk, Connecticut, United States of America. The pH value was done by taking 10 mL of the sample into a beaker. The pH meter was standardized using a buffer solution. The electrode of the pH meter was washed with distilled water and dipped into each beaker with the samples (rinsing out the electrode with distilled water before introducing it into a new beaker). The pH value of the sample was read off.

Total titratable acidity (TTA): The per cent titratable acidity was determined following the method of AOAC⁷. Five grams of the sample was dissolved in a beaker and made up to 100 mL with distilled water and allowed to stand for 30 min. The solution was filtered with Whatman filter paper. Twenty-five millilitres of the filtrate were transferred into a conical flask. Three drops of phenolphthalein indicator were added and titrated with 0.1N NaOH solution:

- Observation; the solution turns pink (red)
- The percent Titrable Acidity (TTA %) was calculated using the following formula as described by AOAC⁷:

$$TTA(\%) = \frac{\text{Titre} \times 0.1 \text{m NaOH} \times 100}{\text{Volume of used}} \quad (3)$$

Determination of microbial load: Each sample was serially diluted using sterile distilled water as the diluent. After this, 9 mL of distilled water was measured out into test tubes using separate sterile pipettes. After measuring and thoroughly mixing 1 mL of the sample, the first test tube was filled. Then 1 mL from the first test tube was pipetted into the second test tube, which already held 9 mL of distilled water, using a separate sterile pipette. Up to the final dilution, or test tube, this process persisted using the same methodology. Using distinct sterile pipettes for each sample and its duplicates, 1 mL of each sample unit from the test tubes was pipetted into sterile petri plates using the pour plate method. The prepared agar was then aseptically put into each petri dish and the plate was gently moved while it remained flat on the bench to mix it. Nutrient agar medium was used for the same process. The plates were then incubated at 37°C for 24 hrs. After incubation, the representative colonies on the plates were subcultured on fresh nutrient agar to obtain pure cultures of the isolates. These pure cultures were then transferred into nutrient agar slants for biochemical identification. Total Viable Counts (TVC), Total Fungi Count (TFC) and Total Coliform Counts (TCC) were analyzed using the pour plate method and measured in Colony Forming Units (CFU/mL). The pour plate method was performed in triplicates

for each sample of a serial dilution and the average microbial growth was determined and recorded. The volume of the inoculum was 1 mL and the dilution factor was 10². The number of colonies was counted and calculated using the following specified formula⁸:

$$CFU(mL) = \frac{N}{V \times D} \quad (4)$$

Where:

- Cfu : Colony forming unit
- N : Mean number of colonies
- V : Volume of inoculum
- D : Dilution factor

RESULTS AND DISCUSSION

Table 3 shows the pH levels of watermelon juice produced with citric acid over 28 days. The pH levels changed as time progressed. The initial pH of the juice was around 4.10 for sample 2 with a process variable combination of 10 g of citric acid concentration, pasteurized for 10 seconds and stored at 0°C. On the other hand, sample 11 with 0.5 g citric acid, pasteurized for 20 seconds and stored at 0 degrees had the highest initial pH of 4.60. As the storage days progressed, the pH of the watermelon juice decreased further. At 7 days, the pH ranged from 4.33 to 4.00. At 14 days, it decreased to a range of 3.93 to 3.50. At 21 days, the pH ranged from 3.26 to 3.45. Finally, at 28 days, the pH ranged from 3.20 to 3.47. The pH values of watermelon juice with no citric acid (sample 22) ranged from 4.00 at 0 days to 4.77 at 28 days. On the other hand, the watermelon juice with commercial citric acid (sample 21) had pH values of 4.00 at 0 days and 2.87 at 28 days. Comparing these pH values to the pH levels of the watermelon juice produced with citric acid extracted from June plum, it was observed that the addition of citric acid decreased the pH of the juice compared to sample 22 (without citric acid). This suggested that citric acid played a crucial role in maintaining the pH stability of the juice and preventing excessive alkalization⁹. Additionally, the pH levels of the control samples with commercial citric acid were relatively stable over time, indicating the effectiveness of commercial citric acid in preserving the pH of the juice. The decrease in pH of the watermelon juice produced with citric acid extracted from June plum over time can be attributed to various factors. One possible factor is the fermentation process. Amanda and Choo¹⁰ conducted a study on fermented watermelon juice and observed that the pH continued to decrease during storage, while the concentration of lactic acid increased. This suggests

Table 3: Determination of pH levels of watermelon juice produced with citric acid extracted from June plum over 28 days

Sample	P	V	C	pH (0 days)	pH (7 days)	pH (14 days)	pH (21 days)	pH (28 days)
1	10	20	0	4.30±0.00 ^h	4.10±0.00 ^e	3.55±0.00 ^{jk}	3.31±0.02 ^{gh}	3.36±0.01 ^{fh}
2	10	10	0	4.10±0.00 ⁱ	4.02±0.00 ^f	3.52±0.03 ^{kl}	3.28±0.03 ^{hi}	3.35±0.00 ^{ghi}
3	5.25	15	15	4.50±0.00 ^g	4.20±0.00 ^c	3.80±0.01 ^{fg}	3.33±0.03 ^{fg}	3.31±0.01 ⁱ
4	5.25	15	15	4.54±0.00 ^e	4.21±0.01 ^c	3.83±0.03 ^{defg}	3.30±0.00 ^{gh}	3.32±0.00 ^{hi}
5	5.25	15	15	4.50±0.00 ^g	4.00±0.00 ^f	3.79±0.02 ^g	3.40±0.00 ^d	3.46±0.01 ^c
6	5.25	15	30	4.50±0.00 ^g	4.00±0.00 ^f	3.82±0.03 ^{defg}	3.37±0.03 ^{de}	3.52±0.03 ^b
7	5.25	15	15	4.56±0.02 ^{cd}	4.12±0.03 ^e	3.50±0.00 ^l	3.30±0.00 ^{gh}	3.53±0.06 ^b
8	0.5	20	30	4.54±0.01 ^e	4.20±0.00 ^c	3.56±0.01 ^l	3.45±0.01 ^c	3.45±0.00 ^{cd}
9	5.25	10	15	4.50±0.01 ^g	4.11±0.00 ^e	3.91±0.02 ^c	3.50±0.01 ^b	3.45±0.00 ^{cd}
10	5.25	15	15	4.50±0.00 ^g	4.15±0.01 ^d	3.93±0.06 ^{bc}	3.30±0.00 ^{gh}	3.37±0.02 ^{efg}
11	0.5	20	0	4.60±0.00 ^a	4.00±0.00 ^f	3.85±0.01 ^{de}	3.30±0.00 ^{gh}	3.47±0.03 ^c
12	0.5	10	0	4.58±0.01 ^b	4.25±0.03 ^b	3.82±0.03 ^{efg}	3.37±0.00 ^{de}	3.40±0.00 ^{ef}
13	10	20	30	4.22±0.03 ⁱ	4.00±0.00 ^f	3.95±0.04 ^b	3.28±0.00 ^{hi}	3.33±0.02 ^{ghi}
14	0.5	15	15	4.56±0.02 ^{cd}	4.03±0.00 ^f	3.60±0.00 ^l	3.23±0.03 ^j	3.33±0.06 ^{ghi}
15	5.25	15	15	4.55±0.00 ^{de}	4.20±0.00 ^c	3.84±0.01 ^{def}	3.30±0.00 ^{gh}	3.35±0.00 ^{ghi}
16	5.25	20	15	4.52±0.00 ^f	4.00±0.00 ^f	3.86±0.02 ^d	3.30±0.00 ^{gh}	3.26±0.02 ⁱ
17	5.25	15	0	4.51±0.00 ^{fg}	4.22±0.02 ^{bc}	3.50±0.00 ^l	3.26±0.01 ^{ij}	3.20±0.00 ^k
18	10	15	15	4.50±0.00 ^g	4.00±0.00 ^f	3.70±0.00 ^h	3.30±0.00 ^{gh}	3.25±0.03 ^j
19	10	10	30	4.51±0.00 ^{fg}	4.12±0.04 ^{de}	3.80±0.00 ^g	3.33±0.00 ^{fg}	3.35±0.00 ^{ghi}
20	0.5	10	30	4.57±0.00 ^{bc}	4.33±0.03 ^a	3.83±0.03 ^{defg}	3.35±0.00 ^{ef}	3.41±0.02 ^{de}
21 (CTRL ⁺)	2.5	20	0	4.00±0.00 ^k	3.64±0.02 ^h	3.21±0.01 ^m	2.77±0.06 ^k	2.87±0.02 ^l
22 (CTRL ⁻)	0	20	0	4.00±0.00 ^k	3.90±0.00 ^g	4.22±0.03 ^a	4.73±0.00 ^a	4.77±0.06 ^a

Values are means of duplicate determinations ± Standard Deviation, Values in the same column bearing different superscripts differ significantly (P 0.05). Sample 21: With commercial citric acid, 22: Control sample without any added citric acid, PVC: Process variable combination-Citric acid concentration (g), Pasteurization time (sec) and storage temp (°C)

that microbial activity, such as the growth of lactic acid bacteria, may contribute to the decrease in pH. Another factor that may influence the pH of the watermelon juice is the presence of citric acid. Citric acid is a common additive used in food preservation due to its acidic properties. Penniston *et al.*¹¹ conducted a study on the quantitative assessment of citric acid in fruit juices and found that lemon and lime juices contain higher levels of citric acid compared to other fruit juices. Therefore, the addition of citric acid from June plum to the watermelon juice may have contributed to the decrease in pH over time. Tarazona-Díaz *et al.*¹² investigated the potential of watermelon juice as a functional drink for athletes and explored the bioavailability of citrulline, a compound found in watermelon juice and its potential benefits for sore muscle relief in athletes. Another study examined the effect of storage temperatures on the physicochemical and phytochemical properties of watermelon juice. It found that storage at different temperatures affected the pH, total soluble solids and antioxidant properties of the juice. The study concluded that lower storage temperatures helped maintain the quality of the juice for a longer period¹³. It is important to note that other factors, such as pasteurization and storage conditions, can also affect the pH of the watermelon juice. Pasteurization is a heat treatment process used to kill microorganisms and extend the shelf life of the juice. A previous study on the effect of high-pressure carbon dioxide and mild heat treatment on watermelon juice,

has shown that the overall quality parameters, including pH, were influenced by the pasteurization process¹⁴. It is important to note that pH is a critical factor in determining the safety and quality of food products. Changes in pH can affect the taste, texture and microbial stability of the product. Therefore, monitoring and controlling the pH levels of produced watermelon juice is essential for ensuring its safety and shelf life¹⁵.

Table 4 shows the TTA levels of watermelon juice produced with citric acid over 28 days. The TTA levels changed as time progressed. The initial TTA level of the juice was 0.72 for sample 14 with a process variable combination of 0.5 g of citric acid concentration, pasteurized for 15 seconds and stored at 15°C. On the other hand, sample 19 with 10 g citric acid, pasteurized for 10 sec and stored at 30°C had the highest initial TTA of 2.46. As the storage days progressed, the TTA of the watermelon juice increased further. At 7 days, the TTA level ranged from 0.93 to 2.38. At 14 days, it increased to a range of 0.92 to 2.58. At 21 days, the TTA level ranged from 0.96 to 2.68. Finally, at 28 days, the TTA ranged from 0.99 to 2.73. The watermelon juice with no citric acid (sample 22) had TTA values of 0.72 at 0 days to 0.97 at 28 days. On the other hand, TTA values of the watermelon juice with commercial citric acid (sample 21) ranged from 1.29 at 0 days to 1.22 at 28 days. It was observed that TTA values of the watermelon juice produced with citric acid extracted from June plum increased the pH of the juice when compared with sample 22 (without

Table 4: TTA levels determinations of watermelon juice produced with citric acid extracted from June plum over 28 days

Sample	P	V	C	TTA (0 days)	TTA (7 days)	TTA (14 days)	TTA (21 days)	TTA (28 days)
1	10	20	0	1.99±0.02 ^{bc}	2.16±0.05 ^d	2.37±0.02 ^c	2.44±0.06 ^c	2.48±0.05 ^c
2	10	10	0	2.19±0.05 ^{ab}	2.26±0.05 ^c	2.58±0.01 ^b	2.63±0.01 ^b	2.68±0.03 ^b
3	5.25	15	15	1.97±0.03 ^c	1.99±0.03 ^e	2.43±0.03 ^c	2.47±0.02 ^c	2.52±0.02 ^c
4	5.25	15	15	1.18±0.05 ^g	1.27±0.04 ^m	1.77±0.02 ^e	1.82±0.04 ^e	1.87±0.03 ^e
5	5.25	15	15	1.24±0.04 ^{fg}	1.31±0.03 ^{lm}	1.85±0.04 ^d	1.93±0.03 ^d	1.97±0.02 ^d
6	5.25	15	30	1.30±0.01 ^{fg}	1.37±0.02 ^{kl}	1.67±0.03 ^g	1.72±0.06 ^f	1.78±0.05 ^f
7	5.25	15	15	1.36±0.06 ^{efg}	1.47±0.04 ^{jk}	1.75±0.03 ^{ef}	1.80±0.02 ^e	1.84±0.02 ^e
8	0.5	20	30	0.84±0.05 ^h	0.89±0.03 ^o	0.95±0.03 ^k	0.99±0.01 ^{jk}	1.01±0.03 ^{jk}
9	5.25	10	15	1.41±0.01 ^{defg}	1.49±0.02 ^{ij}	1.55±0.03 ^h	1.60±0.03 ^g	1.64±0.02 ^g
10	5.25	15	15	1.45±0.03 ^{def}	1.55±0.04 ^{hi}	1.32±0.01 ⁱ	1.37±0.02 ^h	1.43±0.03 ^h
11	0.5	20	0	0.88±0.04 ^h	0.95±0.02 ^o	0.94±0.08 ^k	1.02±0.03 ^{jk}	1.03±0.04 ^{jk}
12	0.5	10	0	0.83±0.02 ^h	0.88±0.02 ^o	0.92±0.07 ^{kl}	1.04±0.06 ^j	1.05±0.09 ^j
13	10	20	30	2.31±0.04 ^a	2.38±0.04 ^b	2.66±0.03 ^b	2.70±0.03 ^g	2.73±0.03 ^b
14	0.5	15	15	0.79±0.03 ^h	0.86±0.04 ^{op}	0.95±0.05 ^k	0.99±0.04 ^{jk}	1.02±0.03 ^{jk}
15	5.25	15	15	1.36±0.05 ^{efg}	1.60±0.04 ^{gh}	1.63±0.04 ^g	1.68±0.05 ^f	1.73±0.03 ^f
16	5.25	20	15	1.54±0.04 ^{de}	1.66±0.03 ^{fg}	1.69±0.02 ^{fg}	1.73±0.01 ^f	1.78±0.02 ^f
17	5.25	15	0	1.63±0.03 ^d	1.72±0.03 ^f	1.76±0.03 ^{ef}	1.80±0.05 ^e	1.85±0.05 ^e
18	10	15	15	2.41±0.02 ^a	2.47±0.05 ^{ab}	2.64±0.03 ^{ab}	2.69±0.01 ^a	2.72±0.02 ^e
19	10	10	30	2.42±0.03 ^a	2.55±0.04 ^a	2.68±0.01 ^a	2.73±0.01 ^a	2.79±0.02 ^b
20	0.5	10	30	0.82±0.05 ^h	0.93±0.04 ^o	0.94±0.05 ^k	0.96±0.05 ^k	0.99±0.04 ^a
21 (CTRL ⁺)	2.5	20	0	1.29±0.55 ^{fg}	1.17±0.02 ⁿ	1.19±0.03 ^j	1.21±0.02 ^j	1.22±0.01 ^j
22 (CTRL ⁻)	0	20	0	0.72±0.13 ^h	0.86±0.09 ^p	0.88±0.09 ^a	0.96±0.06 ^a	0.97±0.05 ^k

Values are means of duplicate determinations ± Standard Deviation. Values in the same column bearing different superscripts differ significantly (P 0.05). Sample 21: With commercial citric acid, 22: Control Sample without any added citric acid, PVC: Process variable combination-citric acid concentration (g), Pasteurization time (sec) and Storage temp (°C) and TTA: Titrable acidity

citric acid). The observed changes in TTA levels over time could be attributed to the citric acid degradation and the formation of other organic acids during storage¹⁶. TTA is the amount of base (usually sodium hydroxide) needed to neutralize the acid in a solution when citric acid is added, it increases overall acidity, resulting in a higher TTA value¹⁷. Similar study was conducted by Tiwari *et al.*¹⁸ who analyzed how TTA in orange juice affects the sonication quality parameters. Similarly, Bozkır and Baysal¹⁹ investigated the preservation of apple juice by using a vacuum microwave evaporator which retained the juice titratable acidity.

Microbial analysis: Three essential microbial parameters were targeted for 7 days, 14 days, 21 days and 28 days: Total Viable Counts (TVC), Total Coliform Counts (TCC) and Total Fungi Counts (TFC) was determined. The total viable count (TVC) of watermelon juice produced with citric acid extracted from June plum is shown in Table 5, after 7, 14, 21 and 28 days of study. The study compared different samples with varying concentrations of citric acid, pasteurization times and storage temperatures. The results showed that Sample 1, which had a citric acid concentration of 10 g, was pasteurized for 20 sec and stored at 0°C, had the lowest TVC at all time points except at day 28 where it was second to the lowest. At 7 days, the TVC was 2.60×10^5 CFU/mL, at 14 days it was 2.64×10^5 CFU/mL, at 21 days it was 2.65×10^5 CFU/mL and at 28 days it was 2.54×10^5 CFU/mL. On the other hand, Sample 8, which had a

citric acid concentration of 0.5 g, was pasteurized for 20 sec, stored at 30°C and had the highest TVC at all time points except for 28 days where it was one of the highest.

Table 6 shows the total Coliform Count of the watermelon juice produced with citric acid extracted from June plum. At 7 days, the TVC was 4.92×10^5 CFU/mL, at 14 days it was 4.96×10^5 CFU/mL, at 21 days it was 4.99×10^5 CFU/mL and at 28 days it was 4.45×10^5 CFU/mL. Also, the control sample 22 (without citric acid) had higher TVC values: 4.90×10^5 CFU/mL at 7 days, 4.94×10^5 CFU/mL at 14 days, 4.98×10^5 cfu/mL at 21 days and 4.77×10^5 CFU/mL at 28 days. Similarly, control sample 21 (with commercial citric acid), had lower TVC values than the the watermelon juice without citric acid and the watermelon juice produced with citric acid extracted from June plum (2.94×10^5 CFU/mL at 7 days, 3.02 CFU/mL at 14 days, 3.02×10^5 CFU/mL at 21 days and 2.27×10^5 CFU/mL at 28 days). The results showed that Sample 1, which had a citric acid concentration of 10 g, was pasteurized for 20 sec and stored at 0°C, had the lowest TVC at all time points except at day 28 where it was second to the lowest. At 7 days, the TVC was 2.60×10^5 CFU/mL, at 14 days it was 2.64×10^5 CFU/mL, at 21 days it was 2.65×10^5 CFU/mL and at 28 days it was 2.54×10^5 CFU/mL. On the other hand, Sample 8, which had a citric acid concentration of 0.5 g, was pasteurized for 20 sec, stored at 30°C and had the highest TVC at all time points except for day 28 where it was one of the highest. These findings suggested that higher concentrations of citric acid,

Table 5: Total Viable Count for watermelon juice produced with citric acid extracted from June plum

Sample	P	V	C	TVC ($\times 10^5$ CFU/mL) (7 Days)	TVC ($\times 10^5$ CFU/mL) (14 Days)	TVC ($\times 10^5$ CFU/mL) (21 Days)	TVC ($\times 10^5$ CFU/mL) (28 Days)
1	10	20	0	2.60 $\pm$ 0.005 ^h	2.64 $\pm$ 0.00 ^k	2.65 $\pm$ 0.05 ^h	2.54 $\pm$ 0.11 ^{jk}
2	10	10	0	2.88 $\pm$ 0.02 ^{fg}	2.94 $\pm$ 0.03 ⁱ	2.67 $\pm$ 0.05 ^h	2.49 $\pm$ 0.07 ^k
3	5.25	15	15	3.76 $\pm$ 0.01 ^{cd}	3.85 $\pm$ 0.01 ^{def}	3.89 $\pm$ 0.09 ^{bcd}	3.30 $\pm$ 0.20 ⁱ
4	5.25	15	15	3.78 $\pm$ 0.02 ^c	3.87 $\pm$ 0.29 ^{cdef}	3.92 $\pm$ 0.05 ^{bc}	3.70 $\pm$ 0.09 ^{efgh}
5	5.25	15	15	3.74 $\pm$ 0.07 ^{cd}	3.92 $\pm$ 0.53 ^{cde}	3.97 $\pm$ 0.03 ^b	3.81 $\pm$ 0.08 ^{ef}
6	5.25	15	30	3.70 $\pm$ 0.08 ^{cd}	3.96 $\pm$ 0.06 ^{cde}	3.98 $\pm$ 0.03 ^b	3.75 $\pm$ 0.10 ^{efg}
7	5.25	15	15	3.71 $\pm$ 0.14 ^{cd}	3.95 $\pm$ 0.55 ^c	3.97 $\pm$ 0.04 ^b	3.86 $\pm$ 0.06 ^e
8	0.5	20	30	4.92 $\pm$ 0.04 ^a	4.96 $\pm$ 0.25 ^{cd}	4.99 $\pm$ 0.02 ^a	4.45 $\pm$ 0.15 ^{cd}
9	5.25	10	15	3.66 $\pm$ 0.02 ^{cd}	3.84 $\pm$ 0.16 ^a	3.88 $\pm$ 0.06 ^{bcd}	3.65 $\pm$ 0.04 ^{fgh}
10	5.25	15	15	3.69 $\pm$ 0.18 ^{cd}	4.70 $\pm$ 0.61 ^{efg}	3.99 $\pm$ 0.01 ^b	3.84 $\pm$ 0.02 ^e
11	0.5	20	0	4.75 $\pm$ 0.04 ^b	4.88 $\pm$ 0.05 ^{ab}	4.95 $\pm$ 0.06 ^a	4.35 $\pm$ 0.05 ^d
12	0.5	10	0	4.80 $\pm$ 0.06 ^{ab}	4.88 $\pm$ 0.03 ^{ab}	4.91 $\pm$ 0.02 ^a	4.50 $\pm$ 0.02 ^d
13	10	20	30	2.64 $\pm$ 0.04 ^h	2.81 $\pm$ 0.08 ^j	2.88 $\pm$ 0.04 ^q	2.69 $\pm$ 0.03 ^j
14	0.5	15	15	4.70 $\pm$ 0.09 ^b	4.85 $\pm$ 0.07 ^b	4.89 $\pm$ 0.09 ^a	4.67 $\pm$ 0.05 ^{ab}
15	5.25	15	15	3.63 $\pm$ 0.07 ^d	3.75 $\pm$ 0.07 ^g	3.81 $\pm$ 0.12 ^{de}	3.59 $\pm$ 0.04 ^{gh}
16	5.25	20	15	3.50 $\pm$ 0.05 ^e	3.64 $\pm$ 0.07 ^h	3.72 $\pm$ 0.09 ^e	3.57 $\pm$ 0.12 ^h
17	5.25	15	0	3.65 $\pm$ 0.08 ^{cd}	3.81 $\pm$ 0.06 ^{fg}	3.86 $\pm$ 0.08 ^{cd}	3.70 $\pm$ 0.05 ^{efgh}
18	10	15	15	2.79 $\pm$ 0.05 ^g	2.83 $\pm$ 0.05 ^j	2.87 $\pm$ 0.07 ^q	2.69 $\pm$ 0.06 ^j
19	10	10	30	2.96 $\pm$ 0.03 ^f	2.96 $\pm$ 0.04 ⁱ	2.98 $\pm$ 0.02 ^f	2.66 $\pm$ 0.23 ^j
20	0.5	10	30	4.72 $\pm$ 0.07 ^b	4.84 $\pm$ 0.05 ^b	4.92 $\pm$ 0.04 ^a	4.58 $\pm$ 0.05 ^{bc}
21 (CTRL ⁺)	2.5	20	0	4.90 $\pm$ 0.10 ^a	4.94 $\pm$ 0.10 ^{ab}	4.98 $\pm$ 0.02 ^a	4.77 $\pm$ 0.05 ^a
22 (CTRL ⁻)	0	20	0	2.94 $\pm$ 0.06 ^f	3.02 $\pm$ 0.07 ⁱ	3.02 $\pm$ 0.07 ^f	2.27 $\pm$ 0.06 ^j

Values are means of duplicate determinations $\pm$ Standard deviation, Values in the same column bearing different superscripts differ significantly (P 0.05). Sample 21: With commercial citric acid, 22: Control Sample without any added citric acid, PVC: Process variable combination-Citric acid concentration (g), Pasteurization time (sec) and Storage temp ($^{\circ}$ C)

Table 6: Total Coliform Count for watermelon juice produced with citric acid extracted from June plum

Sample	P	V	C	TCC ($\times 10^5$ CFU/mL) (7 days)	TCC ($\times 10^5$ CFU/mL) (14 days)	TCC ($\times 10^5$ cfu/mL) (21 days)	TCC ($\times 10^5$ CFU/mL) (28 days)
1	10	20	0	Nil	Nil	Nil	Nil
2	10	10	0	Nil	Nil	Nil	Nil
3	5.25	15	15	0.04 $\pm$ 0.01 ^e	0.04 $\pm$ 0.00 ^e	0.04 $\pm$ 0.01 ^e	0.03 $\pm$ 0.01 ^g
4	5.25	15	15	0.03 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^e	0.03 $\pm$ 0.01 ^g
5	5.25	15	15	0.04 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^g
6	5.25	15	30	0.05 $\pm$ 0.02 ^e	0.05 $\pm$ 0.02 ^e	0.05 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^g
7	5.25	15	15	0.05 $\pm$ 0.03 ^e	0.06 $\pm$ 0.02 ^e	0.06 $\pm$ 0.03 ^e	0.05 $\pm$ 0.03 ^g
8	0.5	20	30	1.37 $\pm$ 0.15 ^c	1.44 $\pm$ 0.17 ^c	1.42 $\pm$ 0.14 ^c	1.33 $\pm$ 0.13 ^{bc}
9	5.25	10	15	0.04 $\pm$ 0.04 ^e	0.05 $\pm$ 0.05 ^e	0.04 $\pm$ 0.04 ^e	0.04 $\pm$ 0.04 ^g
10	5.25	15	15	0.04 $\pm$ 0.03 ^e	0.05 $\pm$ 0.03 ^e	0.05 $\pm$ 0.03 ^e	0.04 $\pm$ 0.02 ^g
11	0.5	20	0	1.67 $\pm$ 0.29 ^{ab}	1.73 $\pm$ 0.29 ^{ab}	1.71 $\pm$ 0.29 ^{ab}	1.36 $\pm$ 0.16 ^b
12	0.5	10	0	1.83 $\pm$ 0.25 ^a	1.89 $\pm$ 0.25 ^a	1.88 $\pm$ 0.26 ^a	1.67 $\pm$ 0.29 ^{bcd}
13	10	20	30	1.43 $\pm$ 0.21 ^c	1.50 $\pm$ 0.19 ^c	1.48 $\pm$ 0.21 ^c	1.28 $\pm$ 0.07 ^{cde}
14	0.5	15	15	1.47 $\pm$ 0.21 ^{bc}	1.54 $\pm$ 0.21 ^{bc}	1.51 $\pm$ 0.21 ^{bc}	1.18 $\pm$ 0.13 ^g
15	5.25	15	15	0.04 $\pm$ 0.02 ^e	0.05 $\pm$ 0.02 ^e	0.04 $\pm$ 0.02 ^e	0.03 $\pm$ 0.01 ^g
16	5.25	20	15	0.05 $\pm$ 0.02 ^e	0.05 $\pm$ 0.02 ^e	0.05 $\pm$ 0.02 ^e	0.03 $\pm$ 0.01 ^g
17	5.25	15	0	0.05 $\pm$ 0.00 ^e	0.05 $\pm$ 0.03 ^e	0.05 $\pm$ 0.03 ^e	0.03 $\pm$ 0.02 ⁱ
18	10	15	15	Nil	Nil	Nil	Nil
19	10	10	30	Nil	Nil	Nil	Nil
20	0.5	10	30	1.37 $\pm$ 0.15 ^c	1.44 $\pm$ 0.15 ^c	1.40 $\pm$ 0.14 ^c	1.14 $\pm$ 0.09 ^{de}
21 (CTRL ⁺)	2.5	20	0	1.30 $\pm$ 0.20 ^c	1.38 $\pm$ 0.20 ^c	1.34 $\pm$ 0.21 ^c	1.09 $\pm$ 0.09 ^e
22 (CTRL ⁻)	0	20	0	0.96 $\pm$ 0.06 ^d	1.02 $\pm$ 0.05 ^d	0.99 $\pm$ 0.07 ^d	0.73 $\pm$ 0.11 ^f

Values are means of duplicate determinations $\pm$ Standard deviation, Values in the same column bearing different superscripts differ significantly (p<0.05). 21, 22: Control sample, PVC: Process variable combination-Citric acid concentration (g), Pasteurization time (sec) and storage temp ($^{\circ}$ C).

longer pasteurization times and lower storage temperatures contribute to lower TVC in watermelon juice. Citric acid is known for its antimicrobial properties¹² and the higher concentration of citric acid in sample 1 likely contributed to its

lower TVC. The effectiveness of citric acid as a preservative is due to its ability to lower the pH of the juice, creating an unfavourable environment for microbial growth. Pasteurization is a common method used to reduce microbial

Table 7: Total Fungi Count for watermelon juice produced with citric acid extracted from June plum

Sample	P	V	C	TFC ($\times 10^5$ CFU/mL) (7 days)	TFC ($\times 10^5$ CFU/mL) (14 days)	TFC ($\times 10^5$ CFU/mL) (21 days)	TFC ($\times 10^5$ CFU/mL) (28 days)
1	10	20	0	0.03 $\pm$ 0.02 ^g	0.04 $\pm$ 0.02 ^l	0.04 $\pm$ 0.02 ^l	0.02 $\pm$ 0.02 ^h
2	10	10	0	0.04 $\pm$ 0.01 ^g	0.19 $\pm$ 0.26 ^{ij}	0.23 $\pm$ 0.31 ^{kl}	0.03 $\pm$ 0.01 ^{gh}
3	5.25	15	15	0.35 $\pm$ 0.13 ^f	0.34 $\pm$ 0.27 ^{ghij}	0.40 $\pm$ 0.12 ^{hij}	0.40 $\pm$ 0.14 ^{ef}
4	5.25	15	15	0.27 $\pm$ 0.18 ^{fg}	0.36 $\pm$ 0.19 ^{efgh}	0.32 $\pm$ 0.18 ^{ijk}	0.30 $\pm$ 0.19 ^{fg}
5	5.25	15	15	0.46 $\pm$ 0.02 ^{ef}	0.57 $\pm$ 0.02 ^{efgh}	0.50 $\pm$ 0.02 ^{ghi}	0.44 $\pm$ 0.04 ^{def}
6	5.25	15	30	0.54 $\pm$ 0.40 ^{def}	0.62 $\pm$ 0.03 ^{defg}	0.55 $\pm$ 0.03 ^{fghi}	0.49 $\pm$ 0.03 ^{cdef}
7	5.25	15	15	0.70 $\pm$ 0.80 ^{cde}	0.71 $\pm$ 0.04 ^{defg}	0.63 $\pm$ 0.11 ^{efgh}	0.52 $\pm$ 0.02 ^{cdef}
8	0.5	20	30	1.07 $\pm$ 0.15 ^{ab}	1.08 $\pm$ 0.08 ^{abc}	1.00 $\pm$ 0.16 ^{abc}	0.62 $\pm$ 0.07 ^{bcd}
9	5.25	10	15	0.78 $\pm$ 0.06 ^{cd}	0.77 $\pm$ 0.03 ^{cdef}	0.75 $\pm$ 0.40 ^{cdefg}	0.80 $\pm$ 0.16 ^b
10	5.25	15	15	0.87 $\pm$ 0.02 ^{bc}	0.84 $\pm$ 0.05 ^{bcd}	0.74 $\pm$ 0.05 ^{cdefg}	0.69 $\pm$ 0.03 ^{bcd}
11	0.5	20	0	0.97 $\pm$ 0.02 ^{abc}	0.97 $\pm$ 0.02 ^{abcd}	0.85 $\pm$ 0.05 ^{cde}	0.72 $\pm$ 0.20 ^{bc}
12	0.5	10	0	1.12 $\pm$ 0.15 ^{ab}	1.08 $\pm$ 0.13 ^{abc}	0.96 $\pm$ 0.04 ^{bcd}	0.52 $\pm$ 0.42 ^{cdef}
13	10	20	30	0.09 $\pm$ 0.01 ^g	0.08 $\pm$ 0.01 ^l	0.07 $\pm$ 0.02 ^{kl}	0.27 $\pm$ 0.34 ^{gh}
14	0.5	15	15	0.43 $\pm$ 0.58 ^f	0.45 $\pm$ 0.61 ^{fghi}	0.90 $\pm$ 1.17 ^{bcd}	0.07 $\pm$ 0.02 ^{gh}
15	5.25	15	15	0.95 $\pm$ 0.03 ^{abc}	0.83 $\pm$ 0.06 ^{bcd}	0.78 $\pm$ 0.04 ^{cdef}	0.85 $\pm$ 0.23 ^b
16	5.25	20	15	0.87 $\pm$ 0.12 ^{bc}	0.85 $\pm$ 0.03 ^{bcd}	0.79 $\pm$ 0.03 ^{cdef}	0.68 $\pm$ 0.03 ^{bcd}
17	5.25	15	0	0.83 $\pm$ 0.12 ^{bc}	0.87 $\pm$ 0.02 ^{bcd}	0.85 $\pm$ 0.02 ^{cde}	0.80 $\pm$ 0.01 ^b
18	10	15	15	0.08 $\pm$ 0.01 ^g	0.08 $\pm$ 0.01 ^l	0.31 $\pm$ 0.42 ^{ijk}	0.67 $\pm$ 0.04 ^{bcd}
19	10	10	30	0.84 $\pm$ 0.05 ^{bc}	0.59 $\pm$ 0.44 ^{efgh}	0.08 $\pm$ 0.01 ^{kl}	0.07 $\pm$ 0.01 ^{gh}
20	0.5	10	30	1.11 $\pm$ 0.12 ^{ab}	1.17 $\pm$ 0.12 ^{ab}	1.12 $\pm$ 0.10 ^{ab}	0.07 $\pm$ 0.01 ^{gh}
21 (CTRL ⁺)	2.5	20	0	1.21 $\pm$ 0.05 ^a	1.28 $\pm$ 0.01 ^a	1.23 $\pm$ 0.02 ^a	1.13 $\pm$ 0.03 ^a
22 (CTRL ⁻)	0	20	0	0.72 $\pm$ 1.10 ^{cde}	0.78 $\pm$ 1.04 ^{cdef}	0.72 $\pm$ 0.09 ^{efgh}	0.69 $\pm$ 0.10 ^{bcd}

Values are means of duplicate determinations $\pm$ Standard deviation, Values in the same column bearing different superscripts differ significantly ($p < 0.05$). 21, 22: Control Sample, PVC: Process Variable Combination-Citric acid concentration (g), Pasteurization time (sec) and Storage temperature ($^{\circ}\text{C}$)

load in food products and the longer pasteurization time in Sample 1 may have resulted in greater microbial reduction. Additionally, lower storage temperatures are known to slow down microbial growth²⁰, which could explain the lower TVC in Sample 1 stored at 0°C compared to Sample 20 stored at 30°C . It is important to note that TVC measures the number of viable microorganisms in a sample, including bacteria, yeast and mould¹⁵. Therefore, under different preservation conditions, the TVC observed in the samples could differ due to differences in the growth and survival of microorganisms. These findings are also consistent with previous studies that have investigated the effect of citric acid on the preservation of watermelon juice. For example, Tarazona-Díaz *et al.*²¹ found that short thermal treatment with citric acid made the bioactive compounds and quality parameters of watermelon juice. Similarly, Perkins-Veazie *et al.*²² studied the effects of chemical and natural additives on the quality and shelf life of cucumber juice. They observed that the absence of preservatives in pure watermelon juice made it susceptible to microbial spoilage but the addition of serendipity berry extract as a preservative reduced microbial load and extended the storage life.

The Total Coliform Count (TCC) of watermelon juice produced with citric acid extracted from June plum was studied throughout 7, 14, 21 and 28 days as shown in Table 6. The results showed that the TCC did not vary among the samples. During the 28 days, TCC was not observed in sample 1, 2, 18 and 19. On the other hand, the highest TCC over the 28

days varied among the other samples respectively and they do not differ significantly ($p < 0.05$). In contrast, sample 22 (without citric acid) had TCC values of 0.96×10^5 CFU/mL at 7 days, 1.02×10^5 CFU/mL at 14 days, 0.99×10^5 CFU/mL at 21 days and 0.73×10^5 at 28 days. Sample 21 (with commercial citric acid), showed TCC values of 1.30×10^5 at 7 days, 1.38×10^5 CFU/mL at 14 days, 1.34×10^5 CFU/mL at 21 days and 1.09×10^5 CFU/mL at 28 days. These findings indicate that the addition of citric acid to watermelon juice can effectively reduce the TCC compared to samples without citric acid. Citric acid has antimicrobial properties and can inhibit the growth of coliform bacteria²². Similar findings have been reported by Mengistu *et al.*²³ who studied the bacteriological quality of locally prepared fresh fruit juice in Eastern Ethiopia and found that 64.1% of the fruit juice samples had a total coliform count higher than the maximum permitted level. Similarly, Chauhan *et al.*¹⁵ observed an increase in total plate counts and yeast and mould count during the storage of sugarcane juice. However, no coliforms were detected in the sugarcane juice beverage. Olu-Taiwo *et al.*²⁴ investigated the microbial quality of sliced pawpaw and watermelon sold in the streets of Accra Metropolis, Ghana. They determined the coliform counts by spreading serial dilutions of the fruit samples on plate count agar, blood agar and MacConkey agar plates. This allowed for the assessment of total aerobic counts and coliform counts.

Table 7 shows the total fungi count of watermelon juice produced with citric acid extracted from June plum. The Total Fungi Count (TFC) of watermelon juice produced with citric

acid extracted from June plum was determined for 7, 14, 21 and 28 days. The samples were differed significantly from each other ($p < 0.05$). The results showed that Sample 1, which had a citric acid concentration of 10 g, was pasteurized for 20 sec and stored at 0°C , had the lowest TFC at all time points. At 7 days, the TVC was 0.03×10^5 CFU/mL, at 14 days it was 0.04×10^5 CFU/mL, at 21 days it was 0.04×10^5 CFU/mL and at 28 days it was 0.02×10^5 cfu/mL. Sample 12 (0.5 g citric acid, pasteurized for 10 sec and stored at 0°C) has the highest fungi count on day 7 and Sample 20 (0.5 g citric acid, pasteurized for 10 sec and stored at 30°C) has the highest TFC as well for day 14 and 21. The TFC results for Sample 12 were 1.12×10^5 CFU/mL at day 7. For Sample 20, the TFC results were 1.17×10^5 CFU/mL at day 14 and 1.12×10^5 CFU/mL at day 28. Sample 9 (5.25 g citric acid, pasteurized for 15 seconds and stored at 15°C) had the highest TFC at day 28 which was 0.8×10^5 CFU/mL. The concentration of citric acid used in the preservation process may have influenced the TFC. Sample 1, which had the lowest TFC had a higher concentration of 10 g citric acid. The significant differences in TFC between samples and controls due to the presence of citric acid (Table 7). Citric acid is known to have antimicrobial properties and can inhibit the growth of fungi²⁵. The addition of citric acid to the watermelon juice may have created an acidic environment that is unfavourable for fungal growth. This is supported by the lower TFC values observed in Sample 1 compared to the control without citric acid. Secondly, the pasteurization time and storage temperature could have affected the TFC. Sample 1, which had a longer pasteurization time of 20 sec, had a higher TFC over 28 days compared to other samples. Pasteurization is a heat treatment process that aims to reduce microbial load, including fungi, in food products¹¹. Additionally, the storage temperature of 0°C in the samples with lower TFC may have slowed down fungal growth, resulting in lower TFC at earlier time points. Furthermore, the composition of the watermelon juice itself could have influenced the TFC. To further support these findings, other studies have investigated the preservation of fruit juices and the effects of different factors on microbial counts. For example, Anumudu *et al.*²⁶ studied the bio-preservative potential of spices in fresh fruit juices and found that watermelon juice made with oshorisho had a fungal load of 1.2×10^6 compared to a control with a load of 2.1×10^7 . In another study, Lani *et al.*²⁷ investigated the chemical and microbiological changes during the fermentation of probiotic watermelon juice and found that lactic acid bacteria count changed with different concentrations of *Lactobacillus paracasei*. Additionally, the antibacterial activity of cinnamaldehyde and clove oil was studied in model food

systems and watermelon juice and found that the composition of natural contaminants in the juice can affect the antibacterial activity of the oils²⁸. Furthermore, the use of *Lactobacillus* species isolated from fermented maize (akamu) was investigated for the bio-preservation of processed watermelon juice, showing the potential to extend the shelf life of the juice and prevent spoilage²⁹.

CONCLUSION

The study has successfully established that the total viable count, total coliform count and total fungi count remained within acceptable limits for up to 28 days when the fruit juice was preserved with citric acid stored at different temperatures. According to Codex standard (CX/NEA 03/16: 2002) the total viable count of any fruit juices should not exceed 3.7-4.7 Log CFU/mL. Referring to this standard, 80% of the juice samples analyzed for TVC had bacteria counts below the recommended values. The result showed that the pH was decreasing (4.10-3.58) while the TTA was increasing (0.82- 2.79) thereby making the watermelon juice more acidic.

SIGNIFICANT STATEMENT

This study investigated the preservative potential of citric acid extracted from June plum on watermelon juice. The study demonstrated that citric acid extracted from June plum can effectively preserve watermelon juice, maintaining microbiological quality for up to 28 days. It established that the total viable count, total coliform count and total fungi count remained within acceptable limits for up to 28 days when the fruit juice was preserved with citric acid stored at different temperature. According to Codex standard (CX/NEA 03/16: 2002) the total viable count of any fruit juices should not exceed 3.7-4.7 Log CFU/ml. Referring to this standard, 80% of the juice samples analyzed for TVC had bacteria counts below the recommended values.

RECOMMENDATION

From the knowledge gained in the course of this research, the following are been recommended:

- That consumers be educated about the benefits of citric acid from June plum as a natural preservative in fruit juices. Providing information on how citric acid helps maintain the nutritional value of the juice and extends its shelf life could boost consumer confidence in these products

- That opportunities should be sought to expand the market for June plum products, both locally and internationally. This might involve collaborating with food producers, processors and retailers to feature June plum-derived products. Also, local farmers should be encouraged to cultivate June plum as a cash crop
- A further study should be carried out to explore the potential of June plum beyond its use as a source of citric acid. Investigate the development of other products, such as June plum jams, jellies, or dried fruit snacks, to utilize the fruit in various forms.

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