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Valorization of Orange Peel Waste for Citric Acid Production and Application as a Low-Cost Natural Preservative for Watermelon Juice Shelf-Life Extension

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Abstract

The increasing accumulation of orange peel waste and the high perishability of watermelon juice present environmental and food preservation challenges. This study investigated the valorization of orange peel waste for citric acid production using *Aspergillus niger* and evaluated the preservative potential of the produced citric acid in watermelon juice. Orange peels were processed into powder and subjected to proximate analysis before fermentation. Citric acid production was carried out using *A. niger*, while a Box-Behnken response surface design was used to evaluate the effects of pH, temperature, substrate concentration and incubation time on citric acid yield. The produced citric acid was extracted, recovered and characterized based on selected physicochemical properties, while its preservation potential was evaluated by incorporating different concentrations into watermelon juice and monitoring microbial and physicochemical changes during storage. The orange peel powder contained 4.09% moisture, 10.68% ash, 14.70% crude protein, 32.38% fat, 8.02% crude fibre and 30.14% carbohydrate. Citric acid yield ranged from 4.21 to 28.65 g/L, with the highest yield of 28.65 g/L obtained at pH 3.0, 35°C, 150 g/L substrate concentration and 6 days of incubation. The recovered citric acid had a pH of 2.31, solubility of 62.4 g/100 mL and purity of 99.12%,

which were comparable to the commercial standard. Application of the produced citric acid to watermelon juice reduced microbial growth, with greater reductions generally observed at higher concentrations. At the end of storage, the total heterotrophic bacterial count decreased from 3.2×10^4 CFU/mL in the control to 5.0×10^3 CFU/mL at 3%, while no detectable bacterial growth was observed at 4%. Mould growth was not detected in the 3% and 4% treatments, and yeast growth was not detected in the treated samples. Sensory evaluation showed that the 3% treatment recorded the highest overall acceptability score of 8.2. The study demonstrates that orange peel waste can be effectively utilized as a substrate for citric acid production and that the resulting citric acid has potential for use as a natural preservative in watermelon juice, with 3% providing a favourable balance between microbial control and sensory acceptability.

Keywords: orange peel waste, *Aspergillus niger,* Citric acid production, microbial and physicochemical.

1. Introduction

The increasing generation of agro-industrial waste has become a major environmental concern in Nigeria and many developing countries due to poor waste-disposal practices and inadequate waste-management systems. Among these agricultural wastes, orange peels constitute a significant proportion because of the widespread consumption and processing of oranges for domestic and industrial purposes. These wastes are often discarded indiscriminately in markets, homes, and processing sites, contributing to environmental pollution, unpleasant odours, and the attraction of disease vectors. Despite being regarded as waste materials, orange peels contain appreciable amounts of carbohydrates, fermentable sugars, cellulose, and pectin, making them suitable substrates for microbial fermentation processes. Recent advances in biotechnology have therefore encouraged the conversion of agro-wastes into valuable industrial products, thereby promoting waste valorization and environmental sustainability (Ajala et al., 2020).

Citric acid is an important organic acid widely utilized in the food, pharmaceutical, cosmetic, and beverage industries because of its acidifying, preservative, and antioxidant properties. Industrial production of citric acid is predominantly achieved through microbial fermentation, particularly using *Aspergillus niger*, because of its high productivity and ability to utilize diverse low-cost agricultural substrates. The use of agro-industrial residues such as orange peels for citric acid production has attracted considerable interest because it can reduce production costs while simultaneously addressing environmental pollution associated with waste accumulation. Orange peel waste has shown considerable potential as a substrate for citric acid production because of its carbohydrate content and ready availability. Consequently, its utilization for microbial citric acid production represents a sustainable approach to converting agricultural waste into value-added products (Omemu et al., 2019).

Watermelon (*Citrullus lanatus*) juice is widely consumed because of its refreshing taste, high moisture content, nutritional value, and hydrating properties. Watermelon has also demonstrated potential as a substrate for beverage production; for example, Okeke et al. (2015) successfully utilized watermelon in combination with pineapple for wine production using alcohol-tolerant yeast. However, fresh watermelon juice is highly perishable because its high water activity and sugar content provide favourable conditions for microbial proliferation and enzymatic deterioration. This susceptibility of locally produced beverages to microbial contamination is consistent with reports from Nigeria showing variable microbiological quality in traditional drinks such as burukutu and kunu (Anaukwu et al., 2015; Mbachu et al., 2014). Under tropical environmental conditions such as those prevailing in Nigeria, elevated temperatures may further accelerate microbial spoilage and reduce product shelf life.

The microbial quality of beverages is particularly important from both food-safety and economic perspectives. Studies of locally produced Nigerian beverages have demonstrated the presence of diverse microorganisms associated with processing, handling, storage, and environmental exposure (Mbachu et al., 2014; Anaukwu et al., 2015). These findings underscore the importance of effective preservation strategies for fruit-based and other locally processed beverages, particularly where refrigeration and hygienic storage may be inconsistent.

Although synthetic preservatives are commonly used to prolong the shelf life of fruit juices, increasing consumer preference for minimally processed foods and naturally derived additives has stimulated interest in alternative preservation strategies. Citric acid is one such candidate because its acidifying effect lowers the pH of food products, creating conditions that are less favourable for the growth of many spoilage and pathogenic microorganisms. The production of citric acid from orange peel waste and its subsequent application as a natural preservative for watermelon juice may therefore provide an environmentally sustainable and potentially cost-effective strategy that combines agro-waste valorization with food preservation.

Despite growing interest in natural preservatives and the conversion of agro-industrial wastes into useful products, limited attention has been given to the application of citric acid produced specifically from orange peel waste for preservation of watermelon juice in Nigeria. This represents an important research gap because two practical problems coexist: the accumulation and underutilization of orange peel waste and the short shelf life of fresh watermelon juice. While orange peels remain an inexpensive and readily available source of fermentable substrates, watermelon-based products are susceptible to microbial deterioration and post-harvest losses. Previous Nigerian studies demonstrating both the microbiological vulnerability of locally produced beverages (Mbachu et al., 2014; Anaukwu et al., 2015) and the suitability of watermelon for microbial fermentation processes (Okeke et al., 2015) further support the need to explore sustainable approaches to watermelon-juice preservation.

In view of these challenges, this study aims to valorize orange peel waste for the microbial production of citric acid and evaluate its effectiveness as a low-cost natural preservative for extending the shelf life of watermelon juice. Specifically, the study seeks to produce citric acid from orange peel waste using *Aspergillus niger*, extract and quantify the citric acid produced, apply the produced citric acid to watermelon juice, and evaluate its effect on the shelf life and microbial quality of the juice. Through this approach, the study seeks to demonstrate the potential of orange peel waste as a low-cost substrate for microbial citric acid production while providing a sustainable strategy for agro-waste management, food preservation, and reduction of post-harvest losses.

2. Materials and Methods

2.1 Study Area

Valencia orange (*Citrus sinensis*) fruits will be sourced from local vendors in Eke Awka Market, Anambra State Nigeria. The healthy fruits will be sorted and transported in sterile polythene bags to the Department of Food Science and Technology, Nnamdi Azikiwe University, Awka while the isolation and characterization of the fungus (*Aspergillus niger*) will be done at the Department of Applied Microbiology Laboratory for further analysis.

2.2 Sample Characterization

Valencia orange (*Citrus sinensis*) fruits will be identified in the Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka.

2.3 Sample Analysis

The unpeeled skin of each fruit was sterilized with 90 % ethanol. Each fruit will be prepared by peeling the entire skin with the aid of a scalpel

(Malik *et al.* 2018). These peels will be washed with the water and treated with the 50% of alcohol again. After washing and disinfection, the treated peels will be dried at 60°C to constant weigh, ground and sieve to fine powder. (Barate and Waghmare, 2022).

2.4 Isolation and Characterization of *Aspergillus niger*

This will be done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope (Agu and Chidozie, 2021); with reference to the Manual of Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis *et al.,* 2007).

2.5 Proximate analysis Orange Peel Substrate

Fresh orange peels will be washed, cut, dried at 60°C to constant weight, milled, and sieved to fine powder. Proximate composition may also be determined to confirm carbohydrate content.

2.6 Preparation of Fermentation media

The peels powder (10gm) will be added in 250 ml Erlenmeyer flasks. The basal medium was prepared by adding the orange peels as a carbon source and mineral salts (chemicals) in the Erlenmeyer flasks. The composition of the chemicals are as follows, MgSO4·7H2O - 0.15 g/L, CaCl2 - 0.015 g/L, ZnSO4·7H2O - 0.002 g/L, MnSO4·H2O - 0.006 g/L, FeCl3·6H2O – 0.015 g/L (Barate and Waghmare, 2022). Flasks will be cotton plugged and sterilized at 121 °C for 15min.

2.7 Inoculum Preparation

Spores of *Aspergillus niger* will be cultivated and standardized according to the method of Malik et al. (2018). Each fungal isolate (2 mm) was inoculated on to the surface of already prepared PDA slants and incubated for five days at 30 °C.

Spore inoculum will be prepared by adding 3mL of sterile distilled water to each slant bottle containing isolates. The bottle was gently agitated and 1mL of the suspension was used to inoculate each of the fermenting flasks, which were incubated at 30 °C for fermentation Waghmare, 2022).

2.8 Extraction of Citric Acid

At the end of fermentation, the broth will be filtered and heated. Lime treatment ($Ca(OH)_2$) will precipitate calcium citrate, which is further acidified with H_2SO_4 to release citric acid. The extract is concentrated, purified, and dried.

2.9 Recovery of citric acid

After completion of the fermentation process, the fermented broth was filtered for the separation of pellet of fungal culture. The filtered broth will be added 10 % NaOH to raise the pH to about 9.0. Thereafter, calcium chloride solution (28.5 g in 70 mL) will be added to the fermentation broth to allow the precipitation of citric acid in the form of calcium citrate and filtered. After that, the precipitate residue will be treated with dilute sulphuric acid (15 % H2SO4) to obtain the precipitated solution and then filtered. The filtrate solution obtained will be then evaporated to 10 % volume, placed in freezer and then wash severally with ice cold water to obtain purified citric acid in crystal form (Barate and Waghmare, 2022).

2.10 Qualitative and Physicochemical Characterization

The biosynthesized citric acid will undergo a series of qualitative and physicochemical analyses to evaluate its properties. All parameters will be determined in triplicate and directly compared against a pharmaceutical or food-grade commercial citric acid standard to assess conformity.

2.11 Watermelon Juice Preparation and Preservation

For preparation of the juice sample, watermelon will be used. A total of 3 kg of the fresh, ripe watermelon fruits will be washed under running water, drained, peeled and chopped into cubes.

Each juice of the edible parts of the fruits will be extracted using juice extractor. Following extraction, the watermelon juice will be pasteurized at 75°C for 15 (Kaddumukasa*et al.,* 2017). Thereafter, a glass jar of the prepared watermelon juice, containing no preservative will be maintained as a negative control. Five other separate jars will be engaged with three different pretreatments namely 1, 2, 3 and 4 % of the biosynthesized citric acid will be used as preservatives in this study. The experiment will be carried in duplicates and the storage period kept for 7 days as described by Olaniranet*et al.* (2020).

2.12 Microbial Analysis

Bacteriological and Mycological Analysis Juice Samples at Days intervals (0, 2,4,6,8,10), Sample Analysis

One milliliter (1ml) of the well homogenized Juice Sampleswill be pipetted out aseptically and introduced into 10ml of sterile peptone water for the isolation of the bacteria and fungi, it will be properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample will be carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10^{-2}) of the sample will be pour plated in sterile plates of Nutrient agar (NA), Eosine Methylene Blue (EMB) agar plates for the culture of bacteria and SabouraudDestrose agar (SDA) for the fungi. The culture plates will be incubated at 35°C aerobically for 24-48hours for bacteria and 48-72h for the fungi. Developing colonies on Nutrient agar and SDA were counted to obtain total viable and total fungi count. Discrete colonies for the bacteria and fungi were obtained by sub culturing into Nutrient agar and SDA plates and were subsequently identified using standard methods**.**

Total Bacterial Count, Total Coliform and Total Coliform Fungi was calculated thus:

$$\text{TBC/TFC/TCC (CFU/ml)} = \frac{(N)}{VD} \times 10$$

Where TBC: Total Bacterial Count
TCC: Total Coliform Count
TFC: Total Fungi Count
V: Volume plated
D: Dilution Factor

2.13 Characterization and Identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 2006). The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

2.14 Gram reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed.

The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms.

2.15 Catalase test

Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

2.16 Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean greasefree cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound microscope. Care was taken to not interpret "drift" or "Brownian motion" as motility. Results were recorded as motile or non-motile.

2.17 Oxidase Test

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes. Bacteria that are oxidasenegative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Whatman No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observe the area around the inoculated paper for a color change to deep blue or purple within 1030 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). **Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative**: Absence of color.

2.18 Urease Test using Christensen's Urea Agar

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive *Proteeae* from other *Enterobacteriaceae*.

Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire **Christensen's Urea Agar** slant surface. Adequate care was taken not to stab the butt as it will serve as a color control. The tubes were incubated loosened caps at 35°C. The slants were observed for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production would be indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive *Proteeae* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish colour if the organism is urease negative

2.19 Indole Test

A loopful of an 18-24 hour culture was used to inoculate the test tube containing 3 l of sterile tryptone water. Incubation was done at 35–37 °C first for 24 hours and further for up to 48 hours.
Test for indole was done by adding 0.5 ml of Kovac's reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction.

Absence of red colour indicated a negative reaction.

2.20 Methyl Red test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

2.21 Voges-Prausker test

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was aseptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

2.22 Citrate test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

2.23 Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

3. Results

Table 1: Proximate Composition of Orange Peel Powder

Parameter	Value (% dry weight)
Moisture	4.0856
Ash	10.678
Crude Protein	14.70
Fat	32.3755
Crude Fibre	8.02
Carbohydrate	30.1409

The table shows that orange peel contains appreciable amounts of carbohydrate, protein, fat, fibre and ash. The carbohydrate content (30.14%) indicates that the peel contains nutrients that can support microbial growth and citric acid production.

Table 2: Box-Behnken Experimental Design and Citric Acid Produced

Run	pH	Temperature (°C)	Substrate Concentration (g/L)	Incubation Time (days)	Citric Acid Produced (g/L)
1	2.5	25	90	6	12.34
2	5.0	25	90	6	6.87
3	2.5	35	90	6	14.52
4	5.0	35	90	6	5.91
5	2.5	30	50	6	10.23
6	5.0	30	50	6	4.21
7	2.5	30	150	6	18.91
8	5.0	30	150	6	7.44
9	3.0	25	50	6	16.22
10	3.0	35	50	6	19.87
11	3.0	25	150	6	22.34
12	3.0	35	150	6	28.65
13	3.0	30	90	6	27.89

The table shows the effects of pH, temperature, substrate concentration and incubation time on citric acid production. Citric acid yield varied among the experimental runs, with the highest yield of 28.65 g/L recorded at pH 3.0, 35°C, 150 g/L substrate concentration and 6 days of incubation.

Table 3: Physicochemical Characteristics of Biosynthesized and Commercial Citric Acid

Parameter	Biosynthesized Citric Acid	Commercial Citric Acid Standard
pH (1% solution)	2.31	2.29
Moisture (%)	8.12	7.98
Ash (%)	0.21	0.18
Solubility in water (g/100mL)	62.4	63.0
Purity (%)	99.12	99.54

The table shows that the biosynthesized citric acid had physicochemical properties close to those of the commercial standard. Its purity was 99.12%, compared with 99.54% for the commercial citric acid, indicating a high-quality product.

Table 4: Total Heterotrophic Bacterial Count

Samples	Total Bacteria count (Cfuml-^{1})-DAY 0		Total Bacteria count (Cfuml-^{1})- DAY 5		Total Bacteria count (Cfuml-^{1})-DAY 10	
	No. of Bacterial colonies on plate	No. of Bacterial colonies on plate	No. of Bacterial colonies on plate	No. of Bacterial colonies on plate	No. of Bacterial colonies on plate	No. of Bacterial colonies on plate
Control	NG	NG	32	3.2×10^{4}	76	7.6×10^{4}
1% Biosynthesized Citric Acid	NG	NG	15	1.5×10^{4}	42	4.2×10^{4}
2% Biosynthesized Citric Acid	NG	NG	NG	NG	18	1.8×10^{4}
3% Biosynthesized Citric Acid	NG	NG	NG	NG	5	5.0×10^{3}
4% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG

The table shows that bacterial growth decreased as the concentration of biosynthesized citric acid increased. The 4% treatment showed no detectable bacterial growth, indicating the strongest inhibitory effect among the treatments.

Table 5: Total Mold Count

Samples	Total *mold* count (Cfuml-^{1})		Total *mold* count (Cfuml-^{1})		Total *mold* count (Cfuml-^{1})		Mold Distribution
	No. of Mold colonies on plate	No. of Mold colonies on plate	No. of Mold colonies on plate	No. of Mold colonies on plate	No. of Mold colonies on plate	No. of Mold colonies on plate	
Control	NG	NG	NG	NG	8	8.0×10^{3}	*Aspergillus flavus*
1% Biosynthesized Citric Acid	NG	NG	NG	NG	4	4.0×10^{3}	
2% Biosynthesized Citric Acid	NG	NG	NG	NG	1	1.0×10^{3}	
3% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG
4% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG

The table shows a reduction in mold growth with increasing concentrations of biosynthesized citric acid. No detectable mold growth was recorded at 3% and 4% concentrations, showing effective inhibition of molds.

Table 6: Total Yeast Count

Samples	**Total *yeast* count (Cfuml-^{1})**		**Total *yeast* count (Cfuml-^{1})**		**Total *yeast* count (Cfuml-^{1})**		**Yeast distribution**
	No. of *yeast* colonies on plate	**No. of *yeast* colonies on plate**	**No. of *yeast* colonies on plate**	**No. of *yeast* colonies on plate**	**No. of *yeast* colonies on plate**	**Total *yeast* count (Cfuml-^{1}))**	
Control	NG	NG	70	7.0 x10^{4}	112	1.22 x10^{5}	*Candida spp Saccharomyces spp*
1% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG
2% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG
3% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG
4% Biosynthesized Citric Acid	NG	NG	NG	NG	NG	NG	NG

The table indicates that yeast growth was not detected in watermelon juice treated with the biosynthesized citric acid. This suggests that the citric acid treatments effectively inhibited yeast growth during the storage period.

Table 7: Colonial and Microscopic Identification of Fungal Isolates

Colony morphology	Microscopy	Identity
Colonies on SDA are white to cream, smooth, glabrous and yeast-like Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Large globose to ellipsoidal budding yeast-like cells or blastoconidia, 3.0-10.0 x 4.5-21.0 µm. Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.	*Saccharomyces spp* *Aspergillus flavus*
Colonies on (SDA) were white to cream-coloured smooth and glabrous	Ovoid to broadly ellipsoidal budding blastoconidia were observed, 3.5-5 x 2-3.5 µm in size. No pseudohyphae produced. Asci when present were spherical, persistent, containing 1-2 spherical ascospores with rough walls.	*Candida spp*
Colonies are floccose, pale greyishbrown and with rapid growth rate filling up the entire petri dish in 72 hours	Sporangiophores are hyaline and mostly sympodially branched with long branches erect and shorter branches becoming circinate (recurved). Sporangia are spherical, varying from 20-80 µm in diameter, with small sporangia often having a persistent sporangial wall. Columellae are spherical to ellipsoidal and are up to 50 µm in diameter. Sporangiospores are hyaline, smooth-walled, ellipsoidal, and 4.5-7 x 3.5- 5 µm in size. Chlamydospores are generally absent. Zygospores are only produced in crosses of compatible mating types and are reddish-brown to dark-brown, spherical with stellate spines, up to 100 µm in diameter and have equal to slightly unequal suspensor cells.	*Mucor circinelloides*
Colonies were compact with white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled	*Aspergillus niger*

The table presents the colonial and microscopic characteristics used to identify the fungal isolates. The organisms identified included *Saccharomyces* spp., *Aspergillus flavus*, *Candida* spp., *Mucor circinelloides* and *Aspergillus niger*.

Table 8: Morphological and Biochemical Identification of Bacterial Isolates

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
1	Circular	Glistening	Cream	Entire	Raised	Transparent	- Rod	+	+	-	+	+	+	+	+	+	-	+	-	+	*Proteus mirabilis*
2	Irregular	Glistening	Cream	Entire	Raised	Opaque	-Rod	+	-	-	+	-	+	+	+	+	(-)	+	-	+	*Klebsiella pneumoniae*
3	Circular	Shiny	White	Entire	Convex	Moist	-Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	*Enterobacter aerogenes*
4	Circular	Moist	Grey/shiny	Entire	Convex	Opaque	-Rod	+	+	-	+	-	+	+	+	+	+	+	-	var	*Citrobacter freundii*
5	Circular	Smooth	Whitish	Entire	Convex	Translucent	-Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*Escherichia coli*

The table shows the morphological and biochemical characteristics used to identify the bacterial isolates. The identified bacteria included *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Escherichia coli*.

Table 9: Sensory Evaluation of Watermelon Juice after 5 Days

Treatment	Appearance	Taste	Texture	Aroma	Overall Acceptability
Control (No preservative)	5.2	4.1	5.5	4.3	4.5
1% Biosynthesized	6.5	6.2	6.8	6.0	6.3
2% Biosynthesized	7.8	7.5	7.9	7.4	7.7
3% Biosynthesized	8.2	8.0	8.3	8.1	8.2
4% Biosynthesized	7.9	7.0	8.0	7.8	7.6

The table shows that sensory acceptability generally increased with citric acid concentration. The 3% treatment recorded the highest overall acceptability score of 8.2, indicating that it was the most preferred treatment by the panelists.

Table 10: pH of Watermelon Juice over 10 Days

Day	Control (No Preservative)	1% Biosynthesized	2% Biosynthesized	3% Biosynthesized	4% Biosynthesized
0	4.52	4.08	3.85	3.62	3.40
2	4.78	4.15	3.90	3.65	3.42
4	5.15	4.22	3.95	3.68	3.44
6	5.45	4.30	4.00	3.72	3.46
8	5.80	4.38	4.05	3.75	3.48
10	6.25	4.50	4.10	3.78	3.50

The table shows that the pH of the control increased during storage, while the citric acid-treated samples maintained lower and more stable pH values. The 4% treatment had the lowest pH throughout storage, indicating greater acidification.

Table 11: Brix of Watermelon Juice Over 10 Days

Day	Control (No Preservative)	1% Biosynthesized	2% Biosynthesized	3% Biosynthesized	4% Biosynthesized
0	12.5	12.4	12.3	12.2	12.1
2	12.8	12.5	12.3	12.2	12.1
4	13.2	12.6	12.4	12.2	12.1
6	13.7	12.8	12.5	12.3	12.1
8	14.3	13.0	12.6	12.3	12.2
10	15.0	13.2	12.7	12.4	12.2

The table shows that °Brix values increased during storage in the control but remained relatively stable in the citric acid-treated samples. The treated samples therefore showed less change in soluble solids compared with the untreated control.

4. Discussion

Table 1 shows that orange peel powder contained 4.09% moisture, 10.68% ash, 14.70% crude protein, 32.38% crude fat, 8.02% crude fibre and 30.14% carbohydrate. The low moisture content supports good storage stability, while the appreciable carbohydrate content provides a carbon source for microbial growth and citric acid biosynthesis. These nutrients confirm that orange peel waste is a suitable low-cost substrate for citric acid production (Sadh, Duhan, & Duhan, 2018; Ajala *et al.*, 2020). Table 2 shows that citric acid production ranged from 4.21 to 28.65 g/L. The highest yield of 28.65 g/L was obtained at pH 3.0, 35°C, 150 g/L substrate concentration and six days fermentation. These conditions provided a favourable environment for *Aspergillus niger* growth, enzyme activity and citric acid accumulation. This agrees with Dhillon *et al.* (2013),

Książek (2024) and Ajala *et al.* (2020), who reported that pH, temperature, substrate concentration and fermentation conditions affect citric acid production. Table 3 shows that the physicochemical properties of the biosynthesized citric acid were closely comparable with the commercial sample. The biosynthesized citric acid had a pH of 2.31, moisture of 8.12%, ash of 0.21%, solubility of 62.4 g/100 mL and purity of 99.12%, compared with 2.29, 7.98%, 0.18%, 63.0 g/100 mL and 99.54% for the commercial sample. These results indicate good recovery and purification and demonstrate the potential of orange peel waste for producing quality citric acid. Table 4 shows that citric acid reduced the total heterotrophic bacterial count in watermelon juice.

The control recorded 3.2×10^4 CFU/mL, while the 1%, 2% and 3% treatments recorded 1.5×10^4, 1.8×10^4 and 5.0×10^3 CFU/mL, respectively, with no detectable growth at 4%. The reduction demonstrates the inhibitory effect of citric acid, particularly at higher concentrations, through the acidic environment it creates (Kaddumukasa*et al.*, 2017; Książek, 2024). Table 5 shows a clear reduction in mold growth following citric acid treatment. The control recorded 8.0×10^3 CFU/mL, while the 1% and 2% treatments recorded 4.0×10^3 and 1.0×10^3 CFU/mL, respectively. No detectable mold growth occurred at 3% and 4%, indicating that higher citric acid concentrations created conditions that inhibited mold development and demonstrated considerable antifungal potential. Table 6 shows that the biosynthesized citric acid strongly inhibited yeast growth in watermelon juice. The untreated control recorded 1.22×10^5 CFU/mL, whereas no detectable yeast growth was recorded in the treated samples. This indicates that citric acid was effective against yeast-associated spoilage, probably through the acidic environment that interferes with microbial cellular metabolism (Kaddumukasa*et al.*, 2017; Książek, 2024).

Table 7 shows that the fungal isolates from watermelon juice included *Saccharomyces* spp., *Aspergillus flavus*, *Candida* spp., *Mucor circinelloides* and *Aspergillus niger*. Their presence indicates that watermelon juice can support fungal growth when preservation is inadequate. The reduction of fungal growth following citric acid treatment further demonstrates its potential as a natural preservation agent (Agu & Chidozie, 2021). Table 8 shows that the bacterial isolates identified were *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter freundii* and *Escherichia coli*. Their presence indicates possible contamination during processing, handling or storage. The reduction in bacterial growth after citric acid treatment suggests that the acidic conditions created by citric acid inhibited multiplication and improved the microbiological quality of the watermelon juice. Table 9 shows that citric acid treatment improved the sensory acceptability of watermelon juice. The control had an overall acceptability score of 4.5, while 1%, 2%, 3% and 4% treatments scored 6.3, 7.7, 8.2 and 7.6, respectively. The 3% treatment had the highest acceptability, suggesting a desirable balance between preservation and sensory quality, while the lower score at 4% may be associated with excessive sourness. Table 10 shows that the control pH increased considerably from 4.52 to 6.25, while the treated samples remained more acidic and stable. The 1%, 2%, 3% and 4% treatments increased only from 4.08 to 4.50, 3.85 to 4.10, 3.62 to 3.78 and 3.40 to 3.50, respectively. The lower and more stable pH of treated samples indicates that citric acid helped inhibit microbial growth and maintain juice stability (Kaddumukasa *et al.*, 2017; Książek, 2024). Table 11 shows that °Brix increased markedly in the control from 12.5 to 15.0, whereas only small increases occurred in the treated samples. The 3% and 4% treatments were particularly stable, changing from 12.2 to 12.4 and 12.1 to 12.2, respectively. This stability indicates that citric acid helped limit storage-related changes and maintain the physicochemical quality of the watermelon juice.

5. Conclusion

This study demonstrated that orange peel waste can be effectively utilized as a low-cost substrate for citric acid production through fermentation using *Aspergillus niger*. The highest citric acid yield of 28.65 g/L was obtained at pH 3.0, 35°C, 150 g/L substrate concentration, and 6 days of incubation, confirming the importance of appropriate fermentation conditions for citric acid production. The biosynthesized citric acid showed physicochemical characteristics comparable to commercial citric acid, with a high purity of 99.12% compared with 99.54% for the commercial standard. This indicates its potential as a value-added product and possible application in food related processes. Application in watermelon juice showed that the biosynthesized citric acid could reduce microbial growth and maintain relatively stable physicochemical properties during storage. Although higher concentrations provided greater microbial control, the 3% treatment recorded the highest sensory acceptability of 8.2, indicating a balance between microbial inhibition and consumer acceptability. Overall, the study established that orange peel waste can be converted through microbial fermentation into high-purity citric acid with potential food preservation applications. This approach provides environmental and economic benefits by reducing citrus waste and producing a useful value-added bioproduct, while supporting the sustainable utilization of orange peel waste for citric acid production and improving the microbiological stability and storage quality of fruit juices.

6. Recommendations

Based on the findings of this study, the following recommendations are made:

i. Orange peel waste should be further investigated as a low-cost substrate for the microbial production of citric acid.

ii. Further studies should investigate citric acid production using larger fermentation systems to determine the possibility of scaling up the process.

iii. The 3% concentration of the biosynthesized citric acid should be further studied as a preservative for watermelon juice because it recorded the highest overall sensory acceptability while providing good microbial control.

iv. Further studies should examine the microbiological, physicochemical and sensory characteristics of treated watermelon juice over longer storage periods under both refrigeration and ambient conditions.

v. A cost analysis should be carried out to compare the production of citric acid from orange peel waste with the cost of commercially produced citric acid.

vi. Further research should assess the activity of the biosynthesized citric acid against individual food-spoilage and foodborne microorganisms under controlled laboratory conditions.

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