## *Original Research Article*

# Physicochemical and Microbiological effect of Citric acid made from Yam peels and *Aspergillus Niger.*


## ABSTRACT

This work was aimed to study the physicochemical and preservative effects of citric acid produced from yam peels using *Aspergillus niger*. Fresh yam peels were washed, sundried and milled into flour, after which it was inoculated with *aspergillus niger* in a nutrient medium and fermented for 6 days at 30℃. Citric acid was extracted from the fermented substrate (1200 mL) and total yield was 418 g of citric acid. Physico-chemical properties of the extracted and commercial citric acid were compared and the results were; pH (2.80 and 2.66), TTA (0.77% and 0.75%) and moisture content (2.40% and 1.8%) respectively. Appearance of both samples were crystalline with the extracted citric acid having a more off white colour. The preservative effects of commercial citric acid and extracted citric acid from yam peels were compared using face centered composite design. A 15 samples of fresh pasteurized watermelon juice were analyzed over a period of 7 days at room temperature with concentrations of 0.2– 0.5% per 100 mL of juice. Microbial analysis (TBC, TCC, and TFC) of citric acid was carried out. For extracted citric acid the results ranged from TBC ($4.40^{c}\times 10^{5}\pm$ 1. 005 CFU/mL to $9.70 \times 10^{5}\pm$ 0.100 CFU/mL), TCC (no growth to $3.90 \times 10^{6}\pm$ 0.05 CFU/mL) and TFC (no growth to $9.70 \times 10^{5}\pm$ 0.100 CFU/mL).While for samples made with commercial citric acid the results ranged from; TBC ($5.30^{b} \times 10^{5}\pm$ 0.001 to $2.65^{b} \times 10^{6}\pm$ 005), TCC (no growth to $8.10 \times 10^{6}\pm$ 0.05 CFU/mL) and TFC (no growth to $2.21 \times 10^{6}\pm$ 0.105 CFU/mL). This implies that citric acid extracted from yam peels had better preservative effects compared to the commercially produced citric acid.



*Keywords: Citric acid, Aspergillus Niger, Yam peel, Physicochemical, Microbiological effect.*


## 1. INTRODUCTION

Citric acid (CA) is a common organic acid utilized in various industries. There is a worldwide demand for citric acid consumption due to their many industrial applications [1]. All microbial cells utilize citric acid as an intermediary in the Tricarboxylic acid cycle (TCA), which is essential to their metabolism [2]. This all-natural component promotes good digestion and kidney function, helps with detoxification, and keeps energy levels stable [3]. It is used to balance the sweetness in soft drinks, juices, and other beverages because of its somewhat tangy and refreshing flavor [4].

Food waste, including leftovers and precooked food, is a biodegradable waste that is released from a variety of sources, such as homes, food processing businesses, and the

hospitality industry. Nearly 1.3 billion tonnes of food, including fresh fruits, vegetables, meat, bread, and dairy products, are wasted within the food supply chain, according to the FAO [5].

According to Lakshmi [6], waste management is an integral part of the supply chains that our global economy relies on. Due to exponential growth of population there has been a remarkable increase in everyday waste wherein improper treatment and disposal cause serious socio-economic downturns. This became a major concern to many of developing countries where safe and sustainable practices are scarce and waste management has not been adequately regulated. However, certain wastes may eventually become resources valuable to others once they are removed from the waste stream [7].

Citric acid exists in a variety of fruits and vegetables. Most notably, citrus fruits, lemons and limes have particularly high concentrations of the acidAccording to Penniston et al. (2008)[8], it can make up as much as 8% of the fruits' dry weight and roughly 47 g/L in their juices.However, under certain conditions of drastic nutrient imbalance, fungi, yeast, and bacteria produce citric acid in excessive amounts [9][10].

Significant attention has been directed towards the proficient use of waste and its management. Several methods of adding value to agricultural waste have been assessed for this aim, taking into account the principles of recycling, pollution management, and reducing environmental littering [11].With the increase in consumption demand, there is need for substrate alternatives in the manufacture of citric acid that is cheaper and available than the present substrate, especially those of our waste products.

Yam peels have several nutritional benefits; they can be used in a variety of food applications; and they can help improve food security in developing countries such as Nigeria. However, their utilization as an industrial raw material is still quite low. In Nigeria, the majority of yam users primarily use the flesh of the crop for year-round basic cooking and use the peels as animal feed, which results in underutilization [12].

The first patent for Citric acid production (CAP) by *Aspergillus niger* utilizing sugar solutions was reported in 1913 [13]. Its high potential for converting various raw materials to valuable products, such as lipase, oxalic acid and microbial lipids, has been demonstrated in previous studies [14][15]. In addition, citric acid and many other products of *A. niger* have been in use for many decades and are considered GRAS (generally regarded as safe) by the U.S. Food and Drug Administration [16].

Yam peels are comprised of mainly carbohydrates with some fractions of protein, fat, and nutrients such as calcium, potassium, magnesium, zinc, and vitamins [17]. The composition of yam peels represents an opportunity to utilize it as a suitable substrate for deriving citric acid via solid state fermentation, which becomes a local means of producing citric acid and reducing Nigeria's dependence on imports to meet her citric acid needs.

It is clear from market trends that there would be a spike in the demand for citric acid globally, as reported by Pau et al. (2015)[18]. There is a rapid increase in the number of food and beverage industries in Nigeria, with little to no known companies generating the acid there. Therefore, it's imperative to maximize the production of citric acid by seeking for substitutes that are more affordable, eco-friendly, and produce more than the existing techniques.

## 2. MATERIAL AND METHODS

### 2.1 Materials Procurement

Yam peels obtained from white yam tubers(*Dioscorea rotundata*) was purchased from Eke-Awka market, Anambra state and the Aspergillus Niger strains was obtained from CognigSimmeone Research LaboratoryAwka, Anambra State.

## 2.2 Preparation of Yam peel

Yam peels were sundried for two weeks followed by size reduction which was done using a grinding machine to turn into powdery form. It was then sieved, packaged in a sterile air tight container, labeled and stored under dry conditions at 30°Cuntil needed for laboratory analyses

## 2.3 Inoculum Preparation

The isolate was incubated in Sabouraud dextrose agar (SDA) at 30°C for 6 days. The inoculums used for citric acid production was prepared by taking 2 fungal plugs with a diameter 8 mm and inoculating a 100 mL broth medium, a modification of Perwitasari*et al.*(2021)[19].

## 2.4 Production of citric acid

Solid state fermentation was carried out using modified methods optimized by Amenaghawon *et al*. (2023)[20]. Firstly, 20 g of the substrate (milled yam peels) was dispensed in a 250 mL Erlenmeyer flask (three flasks were used per experimental run). The substrate was then mixed with a nutrient medium. The composition of the nutrient medium (g/L) was $ZnSO_4$•7 $H_2O$ (0.002), $MgSO_4$•7 $H_2O$ (0.15), $FeCl_3$•6 $H_2O$ (0.015), $CaCl_2$ (0.015), and $MnSO_4$•$H_2O$ (0.006). The flasks were thoroughly shaken to mix the contents and then cotton-plugged before autoclaving at 121°C for 15 minutes to sterilize the samples. The flasks were then cooled and inoculated with the inoculum (2 mL) and incubated for six days at 30°C in a rotary incubator shaker (model: VWR International by B. Bran Scientific and Instrument Company England) at 200 revolutions per minute (rpm). At the end of fermentation the solid substrate was diluted with 100 mL of distilled water and agitated to enhance dissolving process after which it was filtered off using Whatman filter paper and the filtrate used for the analysis.

The concentration of citric acid produced during fermentation was determined using the pyridine acetic anhydride method as reported by Marrier and Boulet (1958)[21]. This was executed by adding 1 mL of the filtered fermentation broth along with 1.30 mL of pyridine and 5.7 mL of acetic anhydride in a test tube. The test tube was then placed in a water bath at 32 °C for 30 min. The absorbance of the sample was measured at 405 nm using a UV-Vis spectrophotometer (PG Instruments model T70). The concentration of citric acid in the sample was determined from a citric acid calibration curve which was prepared from known concentrations of citric acid.

## 2.5 Citric acid Recovery

First, 500 mL of the fermented samples was weighed into a 1000 mL beaker and 500 mL volume of $CaCl_2$ of 40.7% (w/v) was added to the sample, and heated in boiling water bath for 30 minutes. Double displacement reaction takes place between the fermented sample and $CaCl_2$ solution which resulted in the formation of precipitated at the bottom. The resultant mixture was filtered using vacuum filtration and the residue was washed with 100 mL of hot water (100°C) to remove the impurities and by products. During the addition of hot water, the residue was stirred well until it attained a neutral pH of 7. The resultant residue

was then dried in hot air oven. The dried filtrate (calcium citrate) was acidified with 250 mL volume of dilute $H_2SO_4$ of 1.9 M, and heated at $60^0$C with simultaneous mixing with glass rod. When calcium citrate and sulfuric acid were mixed, calcium sulfate precipitated at the bottom leaving behind citric acid solution at the top and the mixture was vacuum filtered. Finally, the citric acid was crystallized from its aqueous solution by evaporative crystallization, the yield of citric acid crystals estimated gravimetrically.

### 2.5 Physicochemical Properties

The moisture, moisture content, pH, yield and total titratable acidity (TTA) of the processed and commercial citric acid was determined using AOAC (2010)[22].

### 2.6 Microbiological Evaluation

The microbiological evaluation of the extracted citric acid was done using the methods of Agu and Chidozie (2021)[23].

### 2.7Statistical Analysis

The mean of all parameters were evaluated for significance (P≤ 0.05) by analysis of variance (ANOVA) and the mean separation and the significant effect tested by Duncan's multiple range of test using SPSS version 23.0

## 3. RESULTS AND DISCUSSION

### 3.1 Physicochemical properties of Citric acid

The physicochemical properties of produced and commercial citric acids are presented in Table 1. From observation the pH values of both samples are acidic, with the produced citric acid having a slightly higher pH of 2.80, and the commercial citric acid having a slightly lower pH of 2.66.
The percentage of acidity (TTA) is slightly higher in the produced citric acid (0.77%) compared to the commercial citric acid (0.75%).
The physical properties of the citric acid derived from the substrate as well as those from commercial source are similar as they were both crystalline and granular in nature, while a slight difference in colour was noticed with the commercial citric being more white and the produced citric acid a more off-white color. The moisture content of the produced citric acid was observed to be slightly higher than that of the commercial citric acid.With produced citric acid having a moisture content of 2.40% while that of the commercial citric acid was observed to be 1.8%. Although the moisture content and color of the fermented citric acid were observed to have improved over the course of time.

**Table 1:Physicochemical properties of citric acid**

| PROPERTIES | Produced citric acid | Commercial citric acid |
|---|---|---|
| Colour | Off-white | White |
| Appearance | Crystal | Crystal |
| pH | 2.80 | 2.66 |

| | | |
|---|---|---|
| TTA (%) | 0.77 | 0.75 |
| Moisture (%) | 2.40 | 1.80 |

## 3.2 Microbial analysisof the Citric acid

The results of the microbial analysis (TBC, TCC and TFC) which was carried out to compare preservative effects of citric acid extracted from yam peels and commercial citric acid are discussed below. The Data points were expressed as means from the triplicate analysis.

**Total Bacteria Count (TBC)**

Table 2 shows the total microbial count (TBC) detected over a period of 7 days. The study compared different samples with varying concentrations of citric acid. The results showed that Citric acid produced from yam peels performed better than commercial citric acid in terms of reducing the TBC of the fruit juice samples. Sample 2 which contained 0.5% of citric acid showed better preservative effects with very little microbial growth up to day 7at $3.10^{a} \times 10^{6} \pm 0.205$ CFU/mL .

This finding was quite similar to the study done by Noor *et al.* (2013)[24] and Jabin *et al*. (2022)[25]. According to the Gulf Standard No. 1016/2000 [26], the maximum bacterial load permitted is $1.0\times10^{4}$ CFU/mL in the case of total viable bacteria. The Microbiological limits in fruit juices and nectars according to UNBS (2009) is maximum of $10^{3}$CFU/g Total Plate Count and 30 CFU/g maximum for yeasts and moulds. This implies that the TBC falls below the standard and the juices cannot be consumed.

These findings are also consistent with previous studies that have investigated the effect of citric acid on the preservation of watermelon juice. For example, El-Saadony*et al*. (2022) [27] studied the effects of chemical and natural additives on the quality and shelf life of the 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 which contains citric acid as preservative reduced microbial loads and extended the storage life. On the other hand sample 3 and sample 5 which had lower concentrations of citric acid had the highest TBC with sample 3 at $1.20^{d}$ x$10^{6}$ CFU/mL which contained citric acid produced from yam peels performing better than sample 5 at $2.65^{b} \times 10^{6} \pm$ 005 CFU/mL which contained commercially produced citric acid at day 7.

**Total Coliform Count**

The total coliform count (TCC) of watermelon juice produced with citric acid extracted from yam peels was studied throughout 7 days as shown in Table 3. The results showed that the TCC did not vary among the samples during the first 6 days except for Control sample which started showing signs of growth at day 4 with $6.80 \times 10^{6} \pm 0.000$.

Sample 1 and 2 showed no observed TCC over the 7days. On the other hand, sample 6 recorded the lowest in numbers at $3.10 \times 10^{5} \pm 0.05$. In contrast, control sample , which had no citric acid added, had TCC values of $6.8\text{x}10^{5}$ CFU/mL at day 4, 9.90 $\text{X}10^{6}$ CFU/mL at day 6, and 121 x$10^{6}$CFU/mL at day 7. It is observed that the samples which contained citric acid produced from yam peels performed better than those with commercial citric acid made with the same concentration of citric acid.

TCC is a measure of the total number of a specific group of bacteria called coliforms in a given sample, typically measured in Colony Forming Units per millilitre (CFU/mL). Citric acid serves as a preservative which limit the growth of microorganisms, in some of the samples like sample 1 and sample 2 there was no observed coliform count. Citric acid is a chelating agent that binds metal ions essential for microbial growth, therefore the citric acid limited the availability of nutrients necessary for prefoliation of the coliform bacterial.

The presence of coliform in fruit juice is not allowed by safe food consumption standard [28]. Total coliform count of this study was higher than the work of Lewis *et al*., (2006) [29] who reported that coliforms counts varied between 0.8-22.2×$10^4$ cfus/100 ml. The Maximum Contaminant Level (MCL) for bacteria in drinking water is zero total coliform colonies per 100 milliliters of water as established by the EPA. From results obtained sample 1 and 2 are the best to prevent coliform growth.

**Total Fungal Count (TFC)**

Table 4. shows the comparison of the total fungi count of watermelon juice produced with citric acid extracted from yam peels and commercial citric acid over a period of 7 days. The samples differ significantly from each other ($p<0.05$).

From the results it is observed that for the first 2 days not much fungal growth was observed, but from day 4 sample 3, 5 and control sample started showing growth this can be attributed to the fact that they contained the least amount of citric acid. The best results were observed in sample 2 which recorded very little growth and had higher amount of extracted citric acid at 0.5 mL. The highest growth by day 7 was observed in sample 3 with 9.7x$10^5$cfu/mL.

From Table 4. the significant differences in TFC among the samples and controls can be attributed to the presence of citric acid. Citric acid is known to have antimicrobial properties and can inhibit the growth of fungi . The addition of citric acid to the watermelon juice may have created an acidic environment that is unfavorable for fungal growth. It was also observed that samples made with citric acid produced with yam peels performed better than those made with commercial citric in the same quantities.

TFC measures the number of fungi or mould in a given sample, typically measured in Colony Forming Units per milliliter (CFU/mL), which showed the presence of fungi within the preserved watermelon juice. The Good Manufacturing Practices (GMP) standard limit for yeasts in fruit juices is <$10^3$ CFU/ml for unpasteurized fruit juices and <10 CFU/ml for pasteurized fruit juices (Development and use of Microbiological criteria for foods, 1997), though the maximum acceptable level is $10^6$ CFU/ml. Based on this standard limit, it can be deduced that the microbial load of the fruit juice samples analyzed are quite high.

**Table 2: Total Bacteria Count TBC (CFU/mL)**

| Sample | Citric acid | TBC ( Day 1) | TBC (Day 2) | TBC (Day 4) | TBC (Day 6) | TBC (Day 7) |
|---|---|---|---|---|---|---|
| 1 | 0.35A | TFTC | $4.70^{d} \times 10^5 \pm 0.100$ | $7.80^{c} \times 10^{55} \pm 0.000$ | $8.40^{b} \times 10^5 \pm 0.110$ | $1.03^{e} \times 10^6 \pm 005$ |
| 2 | 0.5A | TFTC | TFTC | TFTC | TFTC | $3.10^{a} \times 10^6 \pm 0.205$ |
| 3 | 0.2A | $4.40^{c} \times 10^5 \pm 1.005$ | $5.30^{c} \times 10^5 \pm 0.000$ | $6.60^{d} \times 10^5 \pm 1.000$ | $9.70^{a} \times 10^5 \pm 0.001$ | $1.20^{d} \times 10^6 \pm 005$ |
| 4 | 0.35B | $4.20^{d}$ X$10^5 \pm 0.005$ | $6.90^{b} \times 10^5 \pm 0.011$ | $9.20^{b} \times 10^5 \pm 0.005$ | $1.45^{d} \times 10^6 \pm 0.105$ | $1.64^{c} \times 10^6 \pm 005$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | 0.20B | $5.30^{b} \times 10^{5} \pm 0.001$ | $6.10^{b} \times 10^{5} \pm 0.005$ | $1.90^{f} \times 10^{6} \pm 0.000$ | $2.16^{c} \times 10^{6} \pm 0.005$ | $2.65^{b} \times 10^{6} \pm 005$ |
| 6 | 0.5B | TFTC | TFTC | $3.70^{e} \times 10^{5} \pm 0.1$ 05 | $8.40^{a} \times 10^{5} \pm 0.$ 005 | $1.00^{e} \times 10^{6} \pm 005$ |
| ($CTRL^{-}$) | - | $7.80^{a} \times 10^{5} \pm 1.$ 000 | $1.21^{a} \times 10^{6} \pm 0.003$ | $2.63^{a} \times 10^{6} \pm 0.$ 005 | TNTC | TNTC |

Values are means of duplicate determinations ± Standard Deviation. Values in the same column bearing different superscripts differ significantly ($p<0.05$). Citric acid concentration (g), time (days), ),A: Citric acid from Yam peels.B: Commercial citric acid.TFTC: Too Few to Count.TNTC: Too Numerous to Count.TBC: Total Bacteria Count NG: No Growth

**Table 3: Total Coliform Count TCC (CFU/mL)**

| Sample | Citric acid | TCC (Day1) | TCC (Day 2) | TCC (Day 4) | TCC (Day 6) | TCC (Day 7) |
|---|---|---|---|---|---|---|
| 1 | 0.35A | NG | TFTC | TFTC | TFTC | TFTC |
| 2 | 0.5A | NG | NG | NG | TFTC | TFTC |
| 3 | 0.2A | NG | NG | TFTC | TFTC | $3.90 \times 10^{6} \pm 0.05$ |
| 4 | 0.35B | NG | TFTC | TFTC | TFTC | $8.10 \times 10^{6} \pm 0.05$ |
| 5 | 0.20B | NG | TFTC | TFTC | TFTC | $6.20 \times 10^{6} \pm 0.105$ |
| 6 | 0.50B | TFTC | TFTC | TFTC | TFTC | $3.10 \times 10^{5} \pm 0.05$ |
| ($CTRL^{-}$) | - | NG | TFTC | $6.80 \times 10^{6} \pm 0.000$ | $9.90 \times 10^{6} \pm 0.202$ | $1.21 \times 10^{6} \pm 0.105$ |

Values are means of duplicate determinations ± Standard Deviation. Values in the same column bearing different superscripts differ significantly ($p<0.05$). Citric acid concentration (g), time (days), ),A: Citric acid from Yam peels.B: Commercial citric acid.TFTC: Too Few to Count.TNTC: Too Numerous to Count.TBC: Total Bacteria Count NG: No Growth



**Table 4: Total Fungal Count TFC (CFU/mL)**

| Sample | Citric acid | TFC (Day 1) | TFC (Day 2) | TFC (Day 4) | TFC (Day 6) | TFC (Day 7) |
|---|---|---|---|---|---|---|
| 1 | 0.35A | NG | NG | TFTC | $6.10 \times 10^{5} \pm 0.201$ | $7.20 \times 10^{5} \pm 0.005$ |
| 2 | 0.5A | NG | NG | TFTC | TFTC | TFTC |
| 3 | 0.2A | NG | NG | $3.60 \times 10^{5} \pm 0.05$ | $5.20 \times 10^{5} \pm 0.005$ | $9.70 \times 10^{5} \pm 0.100$ |
| 4 | 0.35B | NG | NG | TFTC | $8.20 \times 10^{6} \pm 0.105$ | $1.14 \times 10^{6} \pm 0.001$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | 0.20B | NG | NG | $4.50 \times 10^6 \pm 1.005$ | $7.40 \times 10^6 \pm 0.000$ | $2.21 \times 10^6 \pm 0.105$ |
| 6 | 0.5B | NG | NG | TFTC | $4.90 \times 10^6 \pm 0,003$ | $2.11 \times 10^6 \pm 0.01$ |
| $(CTRL^{-})$ | - | TFTC | TFTC | $8.80 \times 10^6 \pm 0.200$ | $1.24 \times 10^6 \pm 0.05$ | $2.66 \times 10^6 \pm 0.205$ |

Values are means of duplicate determinations ± Standard Deviation. Values in the same column bearing different superscripts differ significantly ($p<0.05$). Citric acid concentration (g), time (days),A: Citric acid from Yam peels.B: Commercial citric acid.TFTC: Too Few to Count.TNTC: Too Numerous to Count.TBC: Total Bacteria Count NG: No Growth

## 4. CONCLUSION

This study revealed that citric acid produced from the fermentation of yam peels can compete favorably with the commercially synthesized citric acid and its utilization will facilitate both the large scale production of this commercially valuable organic acid and also aid in cleaning up of our messed environment, thereby reducing environmental pollution and improving on waste recycling.

The utilization of yam peels for citric acid production could encourage the development of local industries in regions where yam cultivation is prominent. This can lead to the establishment of small-scale processing units, job creation, and the overall development of the agricultural and biotechnology sectors in the area.

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