

## Research Article

# Analysis of Organic Acids in Three Commercial Fruit Juice Samples Sold Across Nigeria

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## Abstract

Fruit juice is one of the highly consumed packaged drinks in Nigeria. As a result of the large consumption and to know the levels of organic acids in them, three (3) fruit juices were selected. The analysis of organic acids in the three fruit juice samples sold within Nigeria was carried out using High Performance Liquid Chromatography (HPLC- Agilent 1260 infinity). A reversed-phase separation using a C-18 column was employed, followed by the organic acids quantification. An isocratic elution method using a buffered mobile phase of  $\text{KH}_2\text{PO}_4$  adequately aided the separation. The organic acids detected comprised oxalic acid, tartaric acid, fumaric acid, ascorbic acid, citric acid, and succinic acid. The results revealed that levels of citric acid in the three samples ranged from 0.23 to 1.07 mg/mL, the levels of ascorbic acid ranged from 0.0016 to 0.203 mg/mL, and the values of oxalic acid ranged from 0.24 to 4.41 mg/mL. As revealed in the results, the organic acids of interest were available in the fruits juices. However, Sample A was the only orange juice that showed a significant amount of tartaric acid recording 0.5 mg/mL. The method demonstrated good linearity, precision, and accuracy making it suitable for routine analysis of juice samples.

## Keywords

HPLC, Organic Acids, Orange Juice, Food Quality, Nigeria, Citric Acid, Oxalic Acid

## 1. Introduction

Fruit juice represents all the essential substances that can be found in the parent fruits when ripe and in good state. It stands as an evolution of the modern and advanced food technology which transfers the essential fruit components into the ex-

tracted juice in order to achieve stable products through physical methods. However, the dietary fibers in the fruits are predominantly sacrificed during the process of pressing, although, fruit purees are exempted from this as they keep virtually the same loads of dietary fibers as contained in original fruit [1].

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The ingestion of beverages in all walks of life is enhanced as a result of their readily availability, convenience, and accessibility. However, over consumption may lead to incidents of overweight and obesity [2]. Following the report of the World Health Organization [3], a dietary pattern underscored by high intake of fatty foods, free sugars, with low consumption of fruits and its derivatives is linked to about 66% surge in productivity loss, also, the risk of obesity and overweight cannot be overemphasized.

Organic acids belong to a group of compounds which are naturally available in various fruits. They play crucial roles in determining the flavours, taste, and the entire quality of fruit juice. These compounds are linked to the acidity and the refreshing tartness inherent from different fruit juices [4]. Organic acids are carbon-structured compounds with acidic properties. They are present in living organisms and commonly found in many fruits such as citrus (lemons and oranges), berries (blueberries, strawberries, etc.), grapes, apples, etc. These acids are responsible for the sensory attributes and fruit juice preservation [5]. Citric acid is no doubt one of the most prevalent and important organic acids in fruit juice. It is abundant in citrus fruits, providing a distinctively sour sensation. It enhances the overall flavour profile of citrus-based juices and serves as a natural preservative. Malic acid is another common organic acid which can be found in cherries, apples, and grapes. Malic acid also contributes to the tartness and fruity flavour of these juices [6]. Tartaric acid is another organic acid that is often present in grape-based juices. It contributes to the sourness and crispness of these juices, adding to their sensory appeal. Although present in smaller quantities than other organic acids, acetic acid can be found in fermented fruit juices. It imparts a tangy taste and is a product of fermentation processes [4]. Analyzing the organic acid composition of fruit juice is vital for the manufacturers to ensure product consistency, quality control and compliance with regulatory standards. It enables them to adjust their products' flavour profile and acidity to meet consumer preferences. Additionally, understanding the organic acid content is valuable for consumers as it helps them make informed choices based on taste preferences and dietary considerations [7].

Although, there a series of publications on fruit juices in Nigeria [8-10], however, not all of them pointed to the organic acids that are embedded in them. So, this study focused on the analysis of three of the most consumed orange juices that are locally sold across virtually every State in Nigeria. As a matter of fact, these three fruit juice samples have become household names that both young and old crave for.

## 2. Materials and Methods

The three different samples of fruit juices (Sample A, Sample B, and sample C) were purchased from a supermarket located within Ado-Ekiti, Nigeria. Standard substances of monobasic potassium phosphate, oxalic acid, tartaric acid, ascorbic acid, fumaric acid, citric acid, and succinic acid were obtained from Sigma-Aldrich. All chemicals and standards used in this study were of analytical grade.

### 2.1. HPLC Method Parameters

The parameters such as mobile phase, analysis time, flow rate, oven temperature, and detection that are needed for the analysis of the juice samples by the HPLC are presented in Table 1.

**Table 1.** HPLC Method Parameters.

Parameter	Description
Column	Agilent 1260 infinity C18, 3.5Mm, 3.5 × 100 mm
Mobile phase	Isocratic, 25 mM K-phosphate buffer, pH 2.4
Analysis Time	15 min
Flow rate	0.3 mL/min (57 bar)
Oven Temperature	20 °C
VW Detection	Wavelength: 210 nm

### 2.2. Preparation of Mobile Phase

A 25 mM phosphate buffer solution used as the mobile phase was prepared by dissolving 3.402 g of potassium phosphate monobasic in 1 L of double distilled water. The pH of the buffer was thereafter adjusted to 2.4 using concentrated ortho-phosphoric acid while monitoring the process with a calibrated pH meter.

### 2.3. Preparation of Standard

A stock standard was prepared from fumaric acid, succinic acid, citric acid, ascorbic acid, tartaric acid, and oxalic acid. The concentrations in mg/mL of the organic acids in the stock standard solution are presented in Table 2.

**Table 2.** Concentration (mg/mL) of organic acids in-stock standard solution.

Organic acids	Oxalic	Tartaric	Ascorbic	Citric	Succinic	Fumaric
Concentration	0.142	0.546	0.162	0.965	0.609	0.021

For calibration purposes, five levels were prepared through serial dilution of the stock standard using double distilled water as diluent. The five levels of serial dilution of the stock standard are presented in Table 3.

**Table 3.** Volume (mL) of the five levels of serial dilutions.

Level	1	2	3	4	5
Stock Standard Solution	0.16	0.32	0.64	1.28	2.56

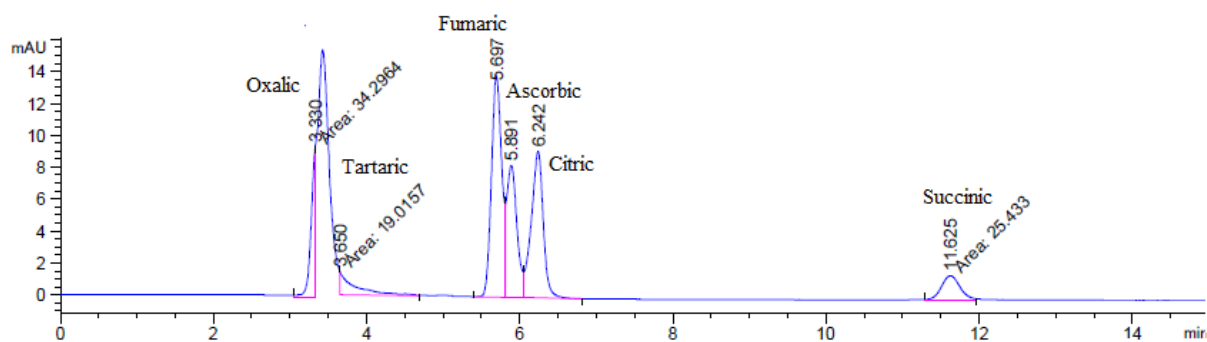
## 2.4. Preparation of Samples

From each juice sample, accurately measured 0.5 mL was

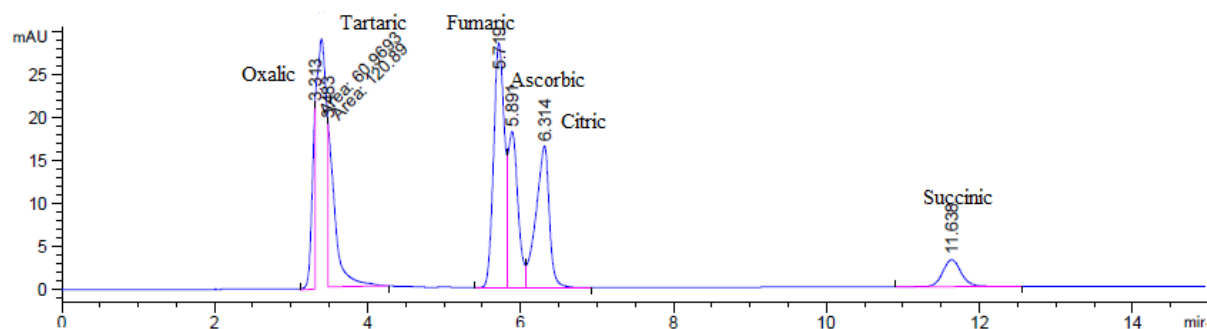
transferred to a 10 mL sample bottle. Double distilled water was added to the sample. The two substances were diluted using ratio 1: 9, the mixture was shaken together, filtered, and then injected into Agilent 1260 infinity HPLC system. The buffered mobile phase, standards, and samples were all filtered through a 0.45  $\mu\text{m}$  filter before injection. Each of the analyses was done in duplicates.

## 3. Results

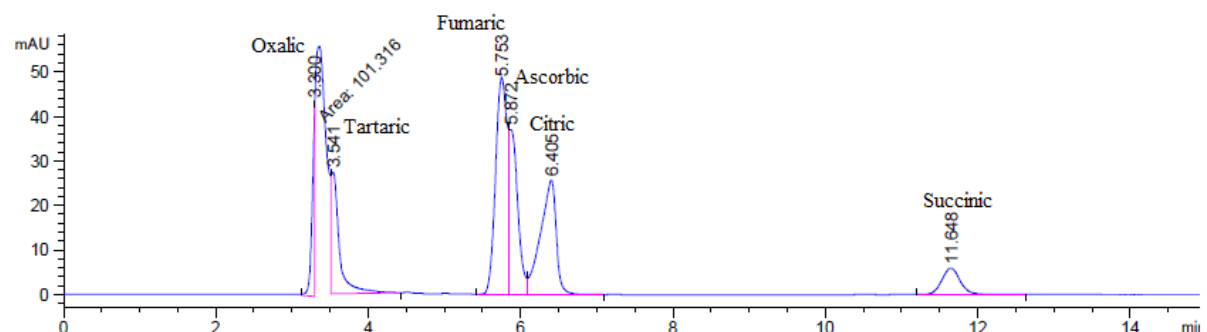
Using the optimized chromatographic conditions, the chromatograms of the stock standards are presented in Figures 1-5.



**Figure 1.** Chromatogram of Standard 1.



**Figure 2.** Chromatogram of Standard 2.



**Figure 3.** Chromatogram of Standard 3.

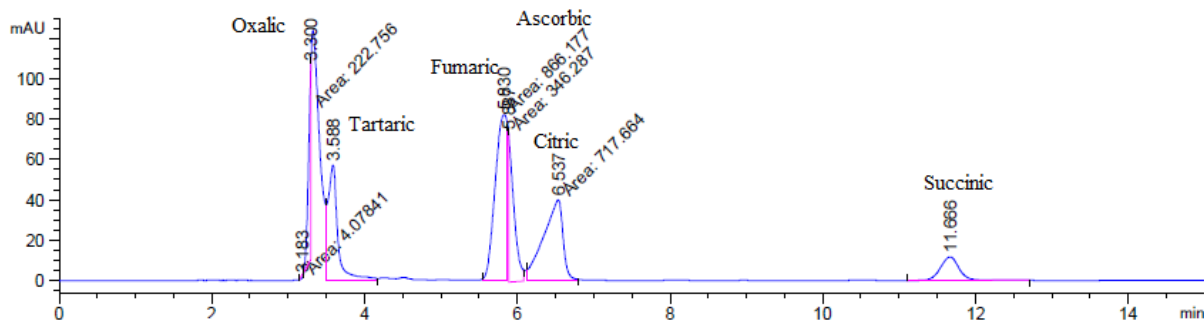


Figure 4. Chromatogram of Standard 4.

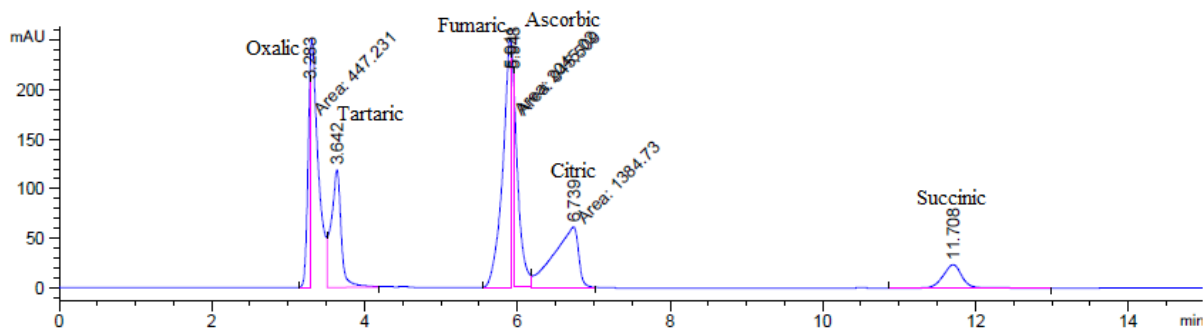


Figure 5. Chromatogram of Standard 5.

From the chromatograms obtained, it was observed that the higher the concentration of the standards, the higher the peak, peak area, and retention time; and the lower the concentration

of the standards, the wider the peak. The resulting chromatograms for the three juice samples as analyzed afterwards are presented in Figures 6-8.

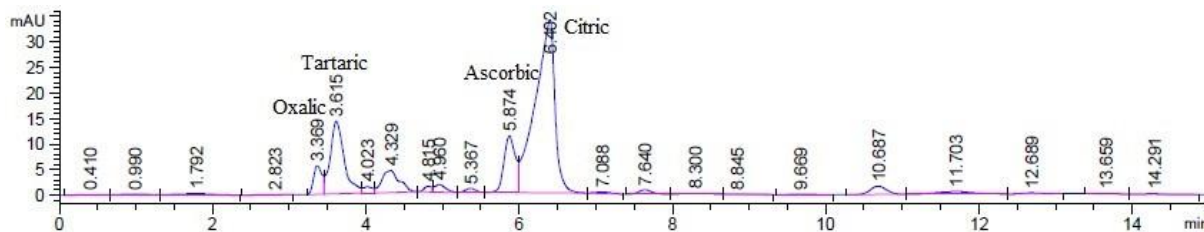


Figure 6. Chromatogram of sample A orange juice.

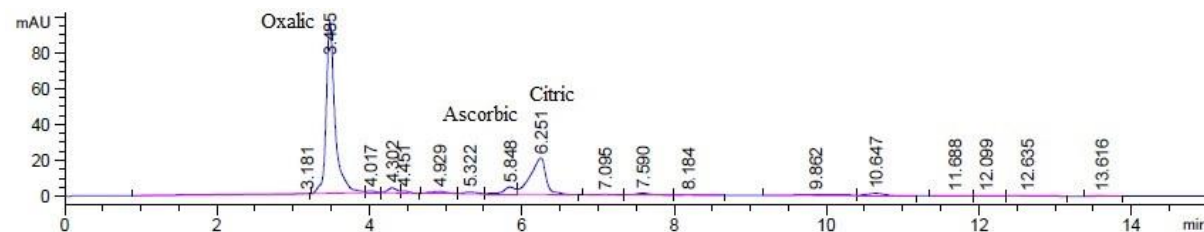
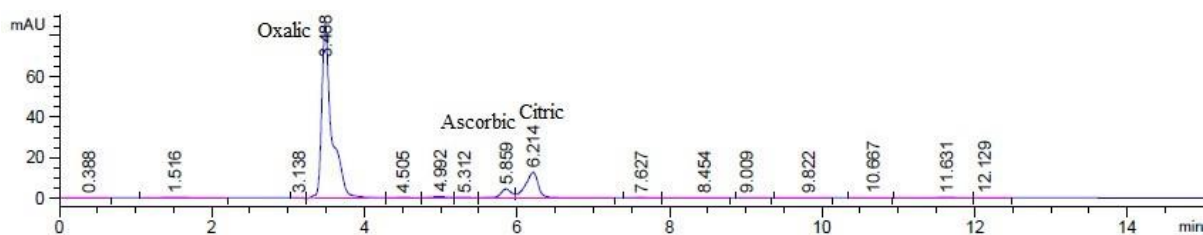


Figure 7. Chromatogram of sample B orange juice.



**Figure 8.** Chromatogram of sample C orange juice.

## 4. Discussion

The individual organic acid contents for each of the three juices are presented in Table 4. Comparing the results obtained for three juice samples, citric acid predominated in sample A orange juice (1.07 mg/mL) while oxalic acid predominated in sample B and sample C orange juices (1.63 and 4.41 mg/mL, respectively). The analysis of organic acids in sample A, sample B, and sample C orange juices revealed important information about the composition of these fruit juices. Interestingly, sample A orange juice was the only juice that showed a significant amount of tartaric acid (0.5 mg/mL). It is worth noting that a high concentration of tartaric acid can be harmful, as it can impart the taste, colour and odour of the final product, it can also cause nausea, vomiting, and abdominal pains in humans. A study by Nour et al. [11] found out that the following amounts of tartaric acid were observed in Minneola (0.376g/L), sweet orange (0.336g/L), and sweetie (0.263g/L).

Samples A, B, and C orange juices contained ascorbic acid (0.203, 0.0060, and 0.0016 mg/mL respectively) and citric acid (1.07, 0.49, 0.23 mg/mL, respectively) which are natural acids found in citrus fruits and are often used to enhance the flavour of juice. Ascorbic acid and citric acid were added as additives in the three juices as shown in their ingredient labels. A study by Higgins et al. [12] suggested that ascorbic and citric acid may be added to those which are naturally present in orange juice to give it more tartness. The ingredient assay in sample B orange juice showed that ascorbic acid was used as an antioxidant in the production of the juice.

Oxalic acid concentrations in sample B and sample C orange juices (1.63 and 4.41mg/mL, respectively) were higher

compared to sample A orange juice (0.24mg/mL). This may be as a result of pre-harvest and postharvest application of oxalic acid which has been known for improving biochemical and physical fruit quality, keeping antioxidant capacity, reducing storage rot and disorders, and down-regulating processes of metabolism [13].

However, none of the juice samples showed any detectable level of fumaric acid. Fumaric acid has a stronger taste than citric acid with a high difficulty to dissolve in water (about 0.6%). Its extended periods, necessary for its solubility into a solution is another concern. So, solubility is most times engineered by heating up the solvent, this is one reason why its use in many food processing industries has been reduced [14].

The results in this study were in consonance with the reports of previous studies on orange-flavoured juices. For instance, a research by Nour et al. [11] pointed out that the levels of ascorbic acid present in some citrus juices ranged from 0.215 to 0.718 g/L. Karadeniz [15] in his own study observed that the citric acid average concentration in orange juice was 13.28 g/L. Ren et al. [16] submitted a report that the concentration of citric acid in juiced oranges was 9.10 g/L. Many factors could have brought some changes in the organic acids concentrations analysed by previous authors. Such factors include the orange variety used [17]; growing conditions [18]; the methods used during processing, for instance, heating of the oranges used could breakdown the citric acid present. Zhang et al. [19] reported processing at both room and low temperature increased sugars accumulation but reduced the citric acid content. Standard or mild pasteurization gently increased the total ascorbic acid content whereas freezing had no significant changes [20].

**Table 4.** Organic acids content (in mg/mL) for Orange Juice samples.

Organic Acid	Sample A	Sample B	Sample C
Oxalic	0.24	1.63	4.41
Tartaric	0.50	ND	ND
Fumaric	ND	ND	ND
Ascorbic	0.203	0.0060	0.0016
Citric	1.07	0.49	0.23

Organic Acid	Sample A	Sample B	Sample C
Succinic	ND	ND	ND

ND: Not detected

## 5. Conclusions

This work demonstrated the effective chromatographic separation and quantification of 6 commonly found organic acids in fruit juice using an Agilent 1260 infinity HPLC system. The analysis of organic acid in the three samples of commercial Juice has revealed distinctive acid profiles in each juice. Citric acid and ascorbic acid emerge as the dominant organic acids in the three orange juice samples while oxalic acid takes precedence in sample B and sample C orange juices. Interestingly, Citric acid, ascorbic acid and oxalic acid are present in all the three samples, indicating its widespread use as a common acidulant in these juices. These findings opened up the unique composition of these drinks, thereby, offering valuable insights into their flavour profiles and potential health benefits. There is a need for constant checking of these organic acids in fruit juice to ensure that they are within the permissible limits of recognized regulatory bodies, this will put a mandate on the manufacturers to maintain recommended standards, and in turn safeguard the health of the final consumers.

## Abbreviations

HPLC	High Performance Liquid Chromatography
ND	Not Detected
WHO	World Health Organization

## Author Contributions

**Olajide Ayodele:** Supervision, Validation, Writing - original draft, Writing - review & editing

**Abiodun Folasade Akinsola:** Project administration, Resources

**Ayomadewa Mercy Olatunya:** Project administration, Resources

**Mayowa Akeem Azeez:** Conceptualization, Data Curation, Formal Analysis

**Edward Olorunsola Olanipekun:** Resources

**Funmilayo Deborah Adewumi:** Project administration, Resources

**Teni Patricia Fagbemi:** Data curation

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