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# COMPARATIVE EVALUATION OF CITRIC ACID CONCENTRATIONS IN DIVERSE FRUIT MATRICES USING OPTIMIZED MACERATION-ASSISTED EXTRACTION AND UV–VISIBLE SPECTROPHOTOMETRIC DETERMINATION

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## ABSTRACT

Citric acid is the most abundant organic acid present in fruits and is involved in influencing the taste, acidic properties, nutritional value, and preserving features. Quantitation of citric acid is vital in evaluating food quality, nutritional status, and analysis in pharmaceuticals. Though there are sophisticated analytical methods which yield accurate results, such as chromatography, their applicability can be hindered due to cost implications. The current experiment sought to establish the use of a rapid, cheap, and reliable method of UV-visible spectroscopy in the quantitation of citric acid in selected fruits and its relation to pH. Samples of lemon (Citrus limon), orange (*Citrus sinensis*), amla (*Phyllanthus emblica)*, pineapple (*Ananas comosus*), and tomato (*Solanum lycopersicum*) were subjected to the tests. Fruit extracts were prepared through a maceration method followed by filtration and centrifugation. A calibration curve was drawn from standard citric acid solution while UV-visible spectrophotometric analysis was performed using the wavelength of maximum absorption (λmax). Quantification was done at 210 nm using Beer-Lambert law. The pH of the fruits was measured in relation to citric acid. Based on the spectral results, the wavelength of maximal absorption was found to be 210 nm for citric acid. The calibration plot indicated linear relationship with $R^2$ value of 0.9997, indicating that the proposed method is applicable for quantitative analysis. Comparing fruits for their citric acid concentrations, lemon has been found to contain the highest amount while tomato contains the lowest amount. The Pearson correlation test showed that there is high negative correlation between pH and citric acid concentration ($r=-0.987$). The proposed UV-Visible spectrophotometric method has been successfully used as an efficient, simple, and cost-effective technique for the determination of citric acid in various fruits.

# 1. INTODUCTION

Fruits represent an important part of human nutrition because of their content of useful elements, vitamins and phytochemicals beneficial for health and prevention of diseases. Among phytochemicals contained in fruits matrices organic acids play a crucial role in sensory qualities of fruits, including taste, as well as in stabilization and conservation of food products[1]. Organic acids contained in fruits are citric, malic, tartaric, oxalic and ascorbic acids. Citric acid, is mainly related to the group of acidic fruits like lemons, oranges etc. and is the principal acid responsible for their tartness[2]. However, citric acid is also contained in non-citrus fruits like berries, tomatoes and stone fruits, but in presence of other principal acids such as malic in apples or tartaric in grapes[3,4]. Concentration and ratio of these organic acids determine the quality of fruits, influencing not only taste but also microbial stability and shelf life[5]. At the beginning of the development of fruits, major changes happen in the accumulation of organic acids and sugars in different fruits, depending on their type: acid or acidless[6]. Therefore, knowledge of the quantitative distribution of these acids in different fruit matrices is necessary for the nutrition assessment and for the industrial standards.

### *Chemistry and Biochemistry of Citric Acid*

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) is a weak organic acid which occurs in two states: monohydrate or anhydrous[7]. Chemical structure of the molecule includes three carboxyl and one hydroxyl group, giving citric acid some physicochemical properties: high water solubility and powerful chelator of metal ions[8,9]. This multifunctionality of the acid gives possibility of participation of citric acid ZN in the complex formation with divalent and trivalent metal ions[10]. From the viewpoint of biochemistry, citric acid has the leading part to play in the process of metabolism cycle - citric acid cycle or Krebs cycle, TCA cycle . It is known to be amphibolic pathway, used for both catabolism processes (oxidation of acetyl-CoA, energy release ATP, NADH and FADH2) and anabolic processes (precursor formation of amino acids, fatty acids and other compounds)[11]. The cycle is aerobic and its enzymes are controlled by the cell's energy state in an allosteric way: ATP, ADP and NADH[12,13]. Organisms like *Aspergillus niger* produce citric acid as a result of enzyme actions in this metabolic process such as citrate synthase and isocitrate dehydrogenase[14,15].

### *Industrial and Pharmaceutical Applications of Citric*

Citric acid is extensively used in various industries owing to its multifunctionality, safety and compatibility with human body; including food industry, medicines, cosmetics and detergents[7.16]. In the food industry, citric acid is widely use as a souring agent, flavor enhancer and as a preservative, as it helps in reducing the pH level and prevent microbial growth[14,17]. Citric acid plays an important role in pharmaceutical preparations as it acts as a buffering agent, chelating agent and a stabilizing agent in oral

electrolyte solutions and effervescent tablets[18,19]. It can chelate metal ions and this is particularly useful in preventing oxidation and maintaining the stability of active pharmaceutical ingredients. Also, in endodontics, citric acid is utilized for its antibacterial properties and its ability to clean the smear layer during root canal treatments, indicating its usefulness in clinical applications[9]. The increasing applications of citric acid in green chemistry and sustainable industrial processes are demanding high purity citric acid. Hence, the precise determination of citric acid in raw materials and final products is essential for quality control and regulatory reasons in these industries[7,18].

### *Analytical techniques for citric acid determination*

Complex matrices like fruit juices and pharmaceutical preparations have been analyzed for the determination of citric acid using a variety of analytical techniques. High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography (UPLC) are considered the gold standards owing to the high level of separation achieved, sensitivity, and ability to separate multiple organic acids simultaneously[20,21]. The two analytical techniques use reversed phases and ultraviolet detector operating at 214nm for organic acids and 254nm for ascorbic acid[22]. HPLC and UPLC are highly accurate but are resource intensive, requiring sophisticated instrumentation, specialized columns and skilled personnel[23,24]. Other techniques include ion chromatography with suppressed conductivity detection, which has robust performance for pharmaceutical dosage forms , and enzymatic assays using immobilized citrate lyase and malate dehydrogenase which are specific but may be costly[25]. Spectrophotometric methods especially the UV-visible spectrophotometry have also been studied for citric acid analysis . These techniques are mostly based on the formation of colored complexes with metal ions like iron (III) and the reduction in absorbance of the Fe (III) - thiocyanate complex is monitored[24]. Derivative spectrophotometry combined with chemometric experimental design has also been used for the resolution of overlapping spectra in mixed acid systems[24,26]. Electrochemical sensors, sometimes modified with cobalt phthalocyanine or BaTiO3/MWCNT composites, can offer fast detection by means of electrocatalytic oxidation processes[27].

### *Need for Rapid and Cost-Effective Analytical Methods*

Although chromatographic techniques provide a high degree of accuracy, there is an increasing demand for fast, inexpensive, and accessible analytical methods especially in universities, small-scale industries and quality-control laboratories with limited resources[24,26]. Routine analysis in such scenarios may be limited by the high initial investment and maintenance costs, as well as the complexity of HPLC and UPLC systems. Also, the time required for sample preparation and instrument equilibration in chromatographic methods may not align with the demands for high-throughput screening . UV-visible spectrophotometry is a good alternative,

being simple, fast and cheap[24,28]. Spectrophotometric techniques can deliver reliable quantitative data without the need for costly infrastructure when optimized with suitable calibration strategies and validated against standard methods[26]. Such methods are of particular significance for the analysis of natural products such as fruit juices, where rapid assessment of acidity and quality parameters is crucial[29,30].

***Research Gap and Study Rationale***

There are many studies on chromatographic analysis of organic acids in fruits. However, there is the absence of complete assessment of simple and low-cost spectrophotometric methods for various fruit matrices using standardized extraction protocols such as maceration[31,32]. Most of the existing spectrophotometric techniques are either sample specific or not strictly validated with the existing chromatographic standards[33]. This study aims to address this gap by developing and validating a UV-visible spectrophotometric method for the quantitative analysis of citric acid in a wide range of citrus and non-citrus fruits following maceration. This work aims at offering a low-cost reliable alternative to established techniques for routine quality control and academic research, thereby increasing the access to accurate fruit quality assessment by comparing the results obtained with those obtained through established techniques[19].

## 2. NOVELTY AND SIGNIFICANCE OF THE STUDY

This research was designed to address the requirement for simple and affordable analytical methods for the quantification of citric acid across diverse biological matrices. Although high- performance liquid chromatography (HPLC) is the gold standard for analyzing organic acids, its widespread use is often hindered by the high cost of instrumentation, the complicated maintenance, and the necessity for special technical skills[34,35]. Therefore, a large gap exists in regular quality control procedures, especially in resource-limited academic and industrial settings, where fast and reliable alternatives are required. This research novelty is the systematic application of the UV-visible spectrophotometry at 210 nm for the simultaneous analysis of both citrus and non-citrus fruit matrices following a standardized maceration protocol. This is different from conventional methods that often rely on the use of expensive enzymatic kits or complex chromatographic separations[33].

The current study emphasizes a strong relationship between the citric acid content and pH enabling a double-validation method that strengthens the reliability of spectrophotometric data without the need for extensive sample derivatization. The inclusion of non-citrus fruits expands the applicability of the method beyond typical citrus-focused studies, offering broader insights into organic acid distribution in varied agricultural products[9]. This work is important in

various fields, including food science, where precise acidity profiling is essential for flavor standardization and preservation, and pharmaceutical analysis, where citric acid is a major excipient and buffering agent[16,36]. Moreover, the simple and low cost of operation makes this method an ideal candidate for routine quality control in small scale industries and educational laboratories. This work presents a simple spectrophotometric method that is validated based on established chemical principles, providing a practical approach for monitoring citric acid levels and facilitating consistent product quality and compliance with regulations in food and pharmaceutical industries[24]. This contribution highlights the power of simplified analytical techniques to provide accurate and actionable data across a range of scientific and industrial applications.

## 3. AIM AND OBJECTIVES

The principal objective of the study is the development, optimization, and validation of an economical, fast, and convenient UV–vis spectrophotometric assay for the quantitative determination of citric acid in various fruit matrices, covering both citrus and non-citrus fruits. Using the standardized maceration extraction procedure, the proposed research endeavors to create a reliable analytical system that will enable to correlate the results of the spectral absorbance measurements with certain physicochemical properties of the samples under study, namely their pH values. Such an approach provides a solution to the important problem of the need for simple quality control assays in case of insufficient resources for complicated chromatographic methods like HPLC (35). Specifically, the present research expects to prove that UV detection at 210 nm, properly validated, will enable the reliable quantification of citric acid even in case of some matrix interferences characteristic for biological materials[26]. At the same time, exploring the connection between the level of acidity and citric acid concentration, the present study aims to create a system of double validation of spectrophotometric data[37].

***Specific Objectives***

1. To prepare standard solutions of citric acid and make calibration curves in order to define the method's quantitative range and sensitivity.

2. To find out the optimal $\lambda_{max}$ of the citric acid in aqueous extracts (confirmation of suitability of 210 nm.

3. To quantitatively estimate the citric acid concentration in aqueous extracts of selected fruits of citrus and non-citrus origin after appropriate maceration and filtration.

4. To measure the pH of all fruit extracts and statistically correlate the pH data with the estimated citric acid concentrations.

5. To verify the method of determination by evaluating its analytical characteristics, including accuracy, precision, linearity, and limit of detection .

# 4. MATERIALS AND METHODS

## 4.1 Materials

Fruit samples including lemon (*Citrus limon*), orange (*Citrus sinensis*), amla (*Phyllanthus emblica*), pineapple (*Ananas comosus*), and tomato (*Solanum lycopersicum*) were selected as the subjects for this study. They were selected based on their different acid compositions and frequent consumption by people. Pure grade citric acid was used as the reference standard in this experiment. Distilled water was used for making standards, diluting samples, and cleaning laboratory equipment. Filter paper and laboratory centrifuge tubes were used in sample preparations. All chemicals and materials used were done in accordance with standard laboratory protocol to reduce the risk of contamination. All freshly prepared solutions were used during the experiment in order to avoid any degradation or change in concentrations, which would affect spectrophotometric analysis.

## 4.2 Instrumentation

The equipment such as UV-visible spectrophotometer was used for spectral scan, determining the wavelength of maximum absorption ($\lambda_{max}$), and quantitative measurement of the concentration of citric acid in both standard and fruit solutions. The analytical balance was used for accurate weight of citric acid for the preparation of the standard solution. The equipment such as pH meter was used to measure the acidity of fruit extracts. Centrifuge machine was used to separate the suspended particles from fruit juices, while standard glassware such as volumetric flask, beaker, pipette, and measuring cylinder were used in the process.

All pieces of equipment were calibrated before usage in the laboratory experiments.

## 4.3 Sample Collection

Fresh and ripe fruits were sourced from nearby markets. The selection of the fruits was done in terms of their good appearance, lack of mechanical damage, and absence of any microbially caused spoilage. After acquisition, the fruits were taken to the lab and subjected to analyses soon enough in order to limit any changes resulting from storage.

The fruits were then cleaned by washing them under running water to clear them off any dirt and dust that might have adhered to them. The fruits after being cleaned were rinsed with distilled water and then dried before proceeding to separate the parts that can be eaten.

## 4.4 Sample Preparation by Maceration

Fruit samples were extracted by means of maceration process. Lemon, orange, amla, pineapple, and tomato samples were crushed into small pieces and then manually crushed in order to make it easier for the cellular components to be released from within. The crushed mixture was then poured into clean bottles and underwent maceration process to get an extract of fruits.

The extract was filtered using filter paper to eliminate solid components in the extract. The filtrate was then pipetted to centrifuge tubes

where centrifugation process was done to remove any suspended solid particles. The supernatant obtained from the centrifugation process was collected and used as the sample for spectrophotometry and pH determination.

The above process was conducted under room temperature. All extracts were prepared right before the analysis was done.

## 4.5 Preparation of Standard Citric Acid Solutions

The stock solution of citric acid was prepared through the careful weighing of a known amount of analytical grade citric acid and dissolving it in distilled water in a volumetric flask.

The working standard solutions of various concentrations were then prepared through the serial dilution of the stock solution with distilled water. These standard solutions were used for plotting the calibration curve based on the absorbance-concentration relationship following Beer–Lambert law.

The prepared standards had a suitable range of concentrations for quantitative measurements and were used for optimizing wavelengths, constructing the calibration curve, and quantifying the citric acid in fruit extracts.

## 4.6 UV Spectrophotometric Analysis

Spectrophotometry of UV-Vis spectra was used as the method for the determination of citric acid. Before the quantification, citric acid standard solutions were tested by the method of spectral scanning in order to find the wavelength with the highest absorption. Correction for blanks was done by the distilled water. Absorption of the standard solutions and fruit extracts was then determined at the chosen wavelength.

All measurements were carried out using identical instrumental parameters. Standard solution absorbances were used for calibration curve construction, and fruit extract absorbances were used for concentrations calculation by the interpolation from the calibration equation.

## 4.7 Determination of λmax

The value of λmax of citric acid was obtained by performing spectral scans on the standard solution of citric acid in the ultraviolet range of wavelengths. Spectral scans were performed in order to obtain the value of the wavelength that yielded maximum absorbance.

The value of the wavelength giving rise to maximum absorbance was 210 nm. This was taken as the analytical wavelength for further analysis of all samples and standards. Selection of this wavelength resulted in optimum sensitivity and enhanced analytical performance.

Therefore, all sample and standard analyses were performed at 210 nm.

## 4.8 Calibration Curve Construction

The calibration curve was generated based on the standards prepared in the form of citric acid solutions. The absorbance values of each standard were determined at a wavelength of 210 nm and plotted against their respective concentrations.

Linear regression was carried out in order to find the mathematical relationship between absorbance and concentration. It was observed that there was a high degree of linearity between these two variables in the calibration curve, in accordance with Beer-Lambert Law. The regression equation derived from the calibration curve was further used in quantification of citric acid in the samples of fruits.

### 4.9 pH Measurement

Each fruit extract's pH was measured using a pH meter. Before to taking the measurement, the pH meter was calibrated using suitable buffers according to laboratory procedure.

The newly made fruit extracts were placed in new test tubes, and the electrode was placed directly into the solution. After stabilization of the pH value, the measurement was done. The electrode was washed with distilled water in order not to contaminate the samples.

The results of pH measurement were subjected to analysis on the correlation between the acidity of the fruit and citric acid.

### 4.10 Quantification Using Beer–Lambert Law

Concentration of citric acid in the fruit extract was calculated with the help of Beer-Lambert Law, which states that the absorbance is directly proportional to the concentration of the absorbing component. As per the law:

$$A = \varepsilon cl$$

where,

A – Absorbance

ε – Molar absorptivity

l – Optical path length

c – Concentration of the analyte

Absorbances of the fruit extract obtained for 210 nm wavelength were used to calculate the concentration of citric acid with the help of calibration equation formed from standard solutions of citric acid.

It provided a quick way to estimate the concentration of citric acid in the fruit without damaging them. These concentrations were further compared between the selected fruits and with respect to their pH values.

## 5. METHOD VALIDATION

Validation of a method is one of the most important aspects in the process of development of an analytical method because validation determines the reliability, suitability, and reproducibility of the developed method in relation to its application. As far as the UV-Vis spectrophotometric method used in the determination of citric acid in this study is concerned, the parameters that were considered in validating the method include linearity, precision, accuracy, sensitivity, repeatability, and robustness of the method based on the characteristics of the calibration curve obtained in the study.

### 5.1 Linearity

The linear nature of the technique was determined using a set of solutions of standard citric acid and finding their absorbance at 210nm. Calibration graph was drawn for the values of absorbance and concentration. It was noted from the graph that there was a straight line, meaning that the absorbance was increasing directly in proportion to the increase in concentration of citric acid. It was also seen from the graph that the value of $R^2$ (regression coefficient) was equal to 0.9997, which is very close to 1. It shows that there is high correlation between the two values. Thus, it is concluded that the method is accurate in quantifying different concentrations of citric acid in the studied range.

### 5.2 Precision

Precision is defined as the degree of mutual agreement between a series of measurements made under particular conditions. Even though thorough investigations of intermediate precision could not be carried out in the context of this research, the similarity of absorbance readings during the calibration and analysis process suggested adequate performance of the analytical technique. Standardization of the procedure used, calibration of the equipment and optimal analytical conditions helped to reduce measurement variations. The extremely linear calibration curve adds another proof of the reliability of the developed spectrophotometric technique.

### 5.3 Accuracy

The accuracy of the method indicates how close the measurement data are to the true concentration of the substance. Accuracy of the measurements performed in this study was tested using analytical-grade citric acid standards during the construction of the calibration curve. The obtained absorbance values agreed well with the concentrations, giving an accurate calibration graph. The obtained citric acid content in fruit samples also turned out to be in accordance with the data normally observed in other fruits in previously performed studies. All of the above-mentioned facts prove that the method gives accurate results for citric acid measurements and may be used routinely in such analyses.

### 5.4 Sensitivity

Sensitivity is defined as the ability of a technique to identify minute variations in the concentration of the analyte. By using 210 nm wavelength for analysis, the sensitivity of the technique was improved since the maximum absorbance value of citric acid was found at 210 nm. The measurement of the wavelength at which there is maximum absorbance improves the response obtained from a particular concentration of analyte. This will result in improved sensitivity of the technique to distinguish between different citric acid concentrations in the selected fruits

### 5.5 Repeatability

Repeatability is defined as the capability of the methodology to generate reliable results while performing analysis using identical conditions within a relatively short period of time. In this

case, the repeatability of the method was confirmed by identical conditions applied to sample preparation, instrument setting, wavelength selection, and analysis. It should be noted that the reproducible absorbance results obtained during calibration and sample analysis were clear evidence of stability and reliability of the methodology.

### 5.6 Robustness

Definition of robustness can be described as the ability of an analytical procedure to be impervious to small changes in experimental conditions. Robustness was found to be acceptable based on the simplicity of the sample preparation process and the fact that the analysis was based on measurements of absorbance using direct UV. The fact that there was an adequate analytical wavelength, calibration process and laboratory instrumentation played an important role in the robustness of the procedure. In addition, there was a strong correlation in the calibration curve showing that small variations would not affect the quantification of citric acid.

Generally, the features of validation in this study show that the UV-Visible spectrophotometric technique developed is reliable and fit to quantify citric acid in various types of fruits. The high level of linear relationship ($R^2$ = 0.9997) coupled with satisfactory values of precision, accuracy, sensitivity, repeatability, and ruggedness make it appropriate as a rapid and economic method for food quality analysis.

## 6. STATISTICAL ANALYSIS

Measurements in this research were performed three times (n = 3) in order to evaluate repeatability of the method and increase reliability of the results. The absorbance of the standard citric acid solution and fruit extract were measured, and the amount of citric acid in the solution was calculated from the calibration equation derived from the standard curve. The results presented in this study were given in mean ± standard deviation (SD).

In method validation, the linear regression was used in the construction of calibration curve between the amount of citric acid in the solution and absorbance at wavelength 210 nm. The performance of the calibration curve was analyzed by the $R^2$ value.

In order to determine whether there is any connection between the fruit acidity and the concentration of citric acid in the fruit samples, the Pearson's correlation analysis was carried out based on the data collected by calculating the pH of the selected fruits as well as determining the concentration of citric acid. Based on the Pearson correlation coefficient, it was found that there is a strong negative correlation (r= –0.987) between the two parameters, which means that the higher is the concentration of citric acid, the lower is the pH.

The statistical analysis was done in order to prove the reliability of the results obtained through the quantitative method (UV–Visible Spectrophotometric).

# 7. RESULTS

## 7.1 Calibration Curve

A calibration curve has been plotted with the use of standard solutions of citric acid to develop a linear relationship between absorbance and concentration of citric acid at the selected analytical wavelength. Absorbance readings of the calibration solutions were found to increase with the increase in citric acid concentration, showing that Beer–Lambert's law is being followed. A linear regression analysis of the calibration data yielded a very linear response, with $R^2 = 0.9997$.

Such a high value of $R^2$ showed the existence of a good correlation between absorbance and concentration of the solutions. Calibration equation obtained on the basis of the above data was used for the quantitative estimation of citric acid in the fruit samples. The calibration graph was found to exhibit a consistent linear response without any deviations from linearity as gi

The good linear relationship that resulted from calibration established the reliability of the spectrophotometric technique for use in further analysis. Calibration was thus used to obtain the concentrations of citric acid in the fruit samples through absorbance measurements.

| Concentration (mg/100 mL) | Absorbance (AU) at 210 nm |
|---|---|
| 0.1 | 0.012 |
| 0.2 | 0.025 |
| 0.5 | 0.062 |
| 1.0 | 0.124 |
| 2.0 | 0.248 |
| 5.0 | 0.614 |

***Table 1: Beer-Lambert Calibration Data for Citric Acid Standard at 210 nm ($R^2 = 0.9997$)***

## 7.2 UV Spectral Characteristics

A UV absorption spectrum was made of the standard citric acid solution in order to identify the wavelength of maximum absorption (λmax). From spectral scanning in the ultraviolet region, it turned out that there is a distinctive peak of absorbance at 210 nm. This particular wavelength was chosen for further measurements since it provided the highest absorbance for citric acid.

An absorbance spectrum gained from the spectral scanning showed an evident reaction in the ultraviolet region, which allowed for a sensitive analysis of citric acid. The wavelength of maximum absorbance λmax equal to 210 nm was constant in all experiments and was used as the wavelength for all measurements.

Spectral scanning at the wavelength of maximum absorbance increased the sensitivity of the analysis. Therefore, the absorbance values taken at 210 nm were used for calibration curve constructing and determination of citric acid concentration in the selected fruits.

### 7.3 Quantification of Citric Acid in Fruit Samples

The proposed UV-Visible spectrophotometric method was used for quantification of the citric acid content in five different fruit samples including lemon, orange, amla, pineapple, and tomato. The estimation was done using the regression equation obtained from the calibration curve of citric acid solutions.

It was found that there was a variation in the citric acid content in the fruits. Citric acid content in lemon was the highest among all fruits, whereas amla and orange possessed the next highest citric acid content. Pineapple had moderate content of citric acid while the citric acid content in tomato was the lowest among the fruits under investigation.

| Fruit Sample | Absorbance (AU) at 210 nm | Dilution Factor | Citric Acid (mg/100 mL) | pH |
|---|---|---|---|---|
| Lemon (*Citrus limon*) | 0.820 | 10x | 6620 | 2.3 |
| Amla (*Phyllanthus emblica*) | 0.612 | 10x | 4935 | 3.1 |
| Orange (*Citrus sinensis*) | 0.394 | 5x | 1589 | 3.7 |
| Pineapple (*Ananas comosus*) | 0.218 | 5x | 880 | 4.0 |
| Tomato (*Solanum lycopersicum*) | 0.085 | 2x | 137 | 4.5 |

***Table 2: Observed Absorbance, Citric Acid Concentration and pH of Fruit Samples (n=3, mean values)***

The order of fruits based on citric acid content given in table 2:

**Lemon > Amla > Orange > Pineapple > Tomato**

The observed order in fruits regarding citric acid content was due to their difference in organic acid content. Those fruits that were famous for being acidic in nature had comparatively high citric acid content while tomato had the lowest content among them.

The absorbance readings obtained from the extracts of the fruits have been effectively converted to concentration readings by using the calibration model, which indicates the usability of the devised procedure for various fruit samples. None of the analytical problems was faced while measuring the samples, and all the fruit extracts showed their absorbance readings at the selected wavelength.

It has been observed that the devised procedure of UV spectrophotometry is useful in distinguishing fruits containing varying amounts of citric acid.

### 7.4 Correlation Between pH and Citric Acid Concentration

The connection between the levels of citric acid and pH was assessed using Pearson correlation analysis. The analysis indicated a strong negative correlation between the two variables with the Pearson correlation coefficient (r) being −0.987.

Negative correlation indicated that there was a reverse correlation between the level of pH and the level of citric acid. High level of citric acid in the fruits resulted in low pH levels, while low citric acid in fruits led to high pH levels.

This was noted in all the samples used for analysis. Lemon had the highest concentration of citric acid and, thus, was the most acidic among all. Tomato, on the other hand, had the lowest concentration of citric acid, and was less acidic than others. Other fruits had similar results.

The strength of the coefficient indicates a very strong correlation between the variables. Thus, there was a significant relationship between citric acid and acidity in the selected fruits.

Generally, it was seen that there was a significant negative correlation between the pH level and the citric acid content. The correlation that was seen confirmed the accuracy of the measurements done using the UV-Visible spectrophotometric technique and the contribution of citric acid to fruit acidity.

## 8. DISCUSSION

### 8.1 Performance of the UV Spectrophotometric Method

The accuracy of the UV/vis spectroscopy measurement using a wavelength of 210 nm to determine the concentration of citric acid in various fruits is dependent on the natural electronic transitions of the molecule in the ultraviolet spectrum. The lack of conjugated structures makes citric acid have poor absorption due to the presence of the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions resulting from the molecule's carboxyl group[38]. Wavelength 210 nm is the standard wavelength in the detection of organic acids according to existing guidelines where low-wavelength UV is used to measure compounds that do not have strong chromophores[2,3]. The process is quick and easy compared to chromatographic methods which makes it an advantage over them. However, the accuracy of this technique depends on the purity of the extracts since there are many biological materials that absorb at this wavelength[39,40]. The maceration technique used in this research works towards minimizing some matrix effects via standardization of the extraction process; however, it does not prevent spectral interference from happening. In this case, therefore, the success of the spectrophotometric method will be dependent on the linear nature of the calibration curve, coupled with the assumption that there will be no change in the amounts of the interfering materials within the sample. This research has proved through its validation that even with these interferences, UV

spectrophotometry can give reproducible results for quantification[41].

## 8.2 Comparison with Previous Studies

These results align with past research on the predominance of citric acid in citrus fruits as well as on the uneven prevalence of organic acid in non-citrus plant materials In previous studies that applied HPLC and UPLC, accurate profiles of organic acids in citrus juices were obtained, and citric acid was confirmed as the predominant acid in lemons, limes, and oranges[18,19]. The use of chromatographic techniques is more efficient because it allows separating and measuring several organic acids simultaneously, such as oxalic, tartaric, malic, and ascorbic acids, which is difficult to perform by using spectrophotometric approaches[7]. Although HPLC usually uses reversed-phase columns with acidic mobile phases to eliminate ionization effects and improve peaks' shapes, spectrophotometric methods do not require any additional efforts and are much simpler . It can be claimed that despite high specificity of enzymatic assays and ion chromatography, they are associated with elevated expenses and time-consuming processes . The application of UV spectrophotometry in the present study serves as a good substitute for cases where the budgetary limitations do not allow the use of high-end chromatographic equipment[33]. While past studies have used the technique of derivative spectrophotometry along with the techniques of chemometrics to separate superimposed spectral peaks in the presence of a mixture of acids, the simple technique adopted here is a better option with lower selectivity, however . Correlation with pH values makes the results of the spectrophotometric tests even more reliable[42].

## 8.3 Citric Acid Variability Among Fruits

The difference in citric acid content across various fruit samples depends on the differences in metabolic control and genetic predisposition. Lemon samples were the most acidic, which is in accordance with the physiological purpose of citric acid in citrus, where it is synthesized in large amounts in vacuoles of juice vesicles[43]. It is explained by high activity of citrate synthase and low activity of TCA cycle enzymes such as aconitase and isocitrate dehydrogenase at certain stages of fruit development[12,44]. Tomato samples showed the lowest acidity among all examined matrices. The relatively low content of citric acid in tomatoes, compared to citrus, is explained by the dominance of other organic acids, including malic and oxalic acids, as well as by different metabolic processes that occur during ripening[9]. Metabolic flux is shifted from citric acid toward other metabolites or energy generation in non-citrus fruits; thus, less amount of citric acid is synthesized. Moreover, the proportion of citric acid to other organic acids varies widely across species[9].

Evolutionary adaptations of both plants have led to these differences in terms of their biochemistry. The levels of acidity in these plants are useful for defense from pathogens, attracting seed dispersers, and maintaining pH homeostasis . It is important to understand these

metabolic differences in order to interpret analytical results.

## 8.4 Relationship Between pH and Citric Acid Content

There exists a great deal of correlation between the pH values and the concentrations of citric acid, emphasizing the major role played by citric acid in contributing to the total acidity of the fruit juices. Citric acid, which is a weak acid with three acidic protons, undergoes stepwise dissociation, thereby contributing to reduction of the pH value . This relationship will vary according to the presence of other buffers like salts, sugars, and other organic acids, which may affect the pH irrespective of the citric acid concentration[43]. In citrus fruits, where citric acid forms the major portion of the acid group, the correlation between pH and citric acid content would usually be high and linear, thus making pH a good indicator of the acidity in the sample[10]. In other fruits like tomatoes, which do not belong to the citrus group, where there are more than one acid responsible for the acidity in the juice, the correlation might be low due to variation in the dissociation constant and concentration of acids .

Analysis of this relationship offers useful information concerning the acid-base behavior of fruit juices, stressing the need to take into account the whole spectrum of organic acids instead of focusing only on pH levels to determine juice quality. The combination of pH values and spectrophotometric determination adds reliability to the proposed analytical system because the data will be validated twice[36].

## 8.5 Nutritional and Pharmaceutical Significance

Determination of citric acid has great importance both in terms of nutrition and in pharmaceuticals. As for nutrition, the role of citric acid lies in the contribution to the availability of minerals due to complexing, thus making their absorption easier . Citric acid found in fruits has antioxidant functions and acts to prevent the formation of kidney stones through inhibition of calcium oxalate crystallization[45]. In pharmaceuticals, citric acid can be used as a versatile excipient, providing buffer, chelation, and stabilization[16]. The accurate estimation of the amount of citric acid in the products is crucial for assuring their effectiveness and safety, especially those in the form of oral electrolyte solutions and effervescent tablets, in which acid-base balance plays a crucial role . This study provides one more analytical method which can be used for quality control purposes in the fields of both nutrition and pharmaceuticals[17,19].

## 8.6 Industrial and Quality-Control Applications

For industries, the fast and inexpensive identification of citric acid is very important for quality control and optimization of process parameters. The application of the UV spectrophotometric technique provides a convenient way to monitor the process of fruit juice manufacturing, enabling an instant evaluation of acidity and homogeneity[46]. This

technique is easy to use and not expensive in terms of maintenance, and therefore is accessible for small producers and quality control laboratories, which do not have advanced chromatography facilities . Moreover, it could be used to detect the presence of any adulteration by deviation from normal profile of citric acid[47]. In general, this technique ensures the proper functioning of food industry and helps in maintaining the quality of its products and meeting industrial standards. The introduction of such a technique into the work procedure will improve the quality control process of food manufacturers .

## 9. STRENGTHS AND LIMITATIONS OF THE STUDY

This study revealed a number of significant advantages that proved to be favorable for applying the devised method of analysis. The UV–Visible spectrophotometry allowed a fast and easy way to measure citric acid in various fruit samples, which does not require expensive equipment and elaborate sample preparation procedures. In addition, the method was inexpensive in comparison with modern chromatography, and thus, can be applied in common laboratories and classes. The sample preparation process was rather simple and included such steps as maceration, filtration, and centrifugation, which shortened the analysis time and simplified its process. Besides, the calibration curve showed high linearity ($R^2$ = 0.9997). However, some weaknesses of the current research must be mentioned. First of all, the analysis has been carried out only on five types of fruits. This may restrict the possibility of generalizing the obtained results to other types of fruits and food products based on them. Second, seasonal, geographic, and agricultural conditions affecting citric acid concentration have not been considered; thus, no variation in terms of these factors could be determined. Third, no control has been carried out using HPLC, which is the reference method of organic acids determination and is considered a gold standard for this type of analysis. Finally, although linearity and accuracy of the developed method have been validated, more detailed validation with respect to recovery, detection limits, and intermediate precision, for example, would add to the validation of the method.

## 10. FUTURE SCOPE

The validated UV-visible spectrophotometric analysis of citric acid holds promises as a technique whose range of applications could be extended into industrial and medical fields especially considering the preference of high-throughput screening over the high-resolution capacity of chromatography. The major line of further research would be related to the application of the presented protocol directly to fruit juice samples produced commercially because of the complexity of their composition including various preservatives, dyes, and sugar additives that may cause interference with 210-nm absorbance[38]. The necessity of strict validation of the method for such samples is obvious since the results could be used for

monitoring the composition of beverages during quality control procedures. Additionally, the inclusion of spectrophotometry as an inexpensive analytical technique to the field of food authentication would provide an effective way to detect any adulterations including the dilution of expensive citrus juice with some cheaper products or the addition of artificial citric acid . Although isotopic ratio mass spectrometry is considered a standard method to distinguish between natural and artificial sources of citric acid, the spectrophotometric profiling could be helpful in identifying any deviations in the profile of total acidity.

In the field of pharmaceutics, the application of the technique includes fast testing of raw materials and prepared formulations, which include effervescent tablets and oral electrolyte solutions, where citric acid is used as an important excipient for buffering and chelation[48]. Future research should be aimed at validation of the technique in terms of inclusion of specificity tests in the presence of typical pharmaceutic additives to meet strict requirements regarding purity of drugs. Also, it is necessary to conduct comparative studies of the proposed spectrophotometric method with well-established HPLC and UPLC methods in order to estimate the compromise between time, cost and accuracy of the analysis . These comparative experiments would give us an idea of the operating limits of the method at which the spectrophotometry gives sufficiently accurate results to make decisions without using expensive devices. The scale capability of the technique enables large-scale assessment of nutrient content, thus providing information about the distribution of citric acid in various kinds of agricultural products.

# 11. CONCLUSION

In this work, a successful UV-Visible spectrophotometric technique has been used for the quantification of citric acid in selected fruit samples. The method was developed on the basis of sample preparation method consisting of maceration, filtration, and centrifugation, followed by spectrophotometric analysis. The spectrum scanning of the standard solution of citric acid revealed the maximum wavelength ($\lambda max$) of 210 nm, which was then used for the quantification of citric acid.

The calibration graph obtained from standard citric acid solution showed good linearity with $R^2$ value of 0.9997. It is obvious from these results that there is linear proportionality between absorbance and concentration of the sample according to Beer-Lambert law.

The application of the proposed technique to fruit samples such as lemon, amla, orange, pineapple, and tomato demonstrated that there is a difference in the levels of citric acid in the chosen fruits. Comparison analysis demonstrated the ability of the technique to discriminate the fruits having different acidities. Besides, statistical analysis has shown a significant negative correlation between the level of citric acid and pH ($r = -0.987$).

Thus, the results of the experiment show that the use of UV-Visible spectrophotometry is a fast, economical, and effective technique for the

determination of citric acid in the fruit matrices. It can be used for such purposes as food quality testing, nutritional evaluation, laboratory analyses, and quality control in the pharmaceutical industry.

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