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RESEARCH PAPER

Effect of Storage Conditions on Citric Acid Content of Beverages Available in Erbil Local Markets

Srwa R. Hamza[1]

[1]Department of Food Technology, College of Agriculture Engineering Sciences, Salahaddin University-Erbil, Kurdistan Region, Iraq

ABSTRACT:

Citric acid has antimicrobial and acidulate properties that support other ingredients' antioxidant benefits and enhance the flavors of syrups, soft drinks, and juices, citric acid is used as a multi-functional food additive in the manufacturing of many foods. Poor storage leads to chemical and physical changes in food and beverages. The effect of temperature and sunlight on storage is considered one of the most important studies in the field of food. The effect of both factors on the concentration of citric acid in beverages available in local markets was studied. Here in, a spectrophotometric method for citric acid determination in beverages was proposed. The basis for the determination is citric acid's inhibitory effect on the thiocyanate – ferrate ion complexation reaction. Citric acid forms complexes with Fe(III), therefore, a decrease in absorbance of the complexation product is monitored at 477 nm. The recommended method's numerous chemical and physical parameters were optimized, and interfering species were also investigated. The calibration graph was constructed in the linear range from 1.0 to 50 mg/L of citric acid with a relative standard deviation of 0.87%, and the results are compared to those obtained from Boehringer–Mannheim ultraviolet method test combination kit for citric acid with an error between -0.51 and 1.86%. The citric acid in commercial beverages has been successfully determined using the approach, which was shown to be sufficiently selective. The results showed that incorrect storage of beverages at 45 ℃ leads to a decrease in the concentration of citric acid from 1999 to 1778, 1930 to 1815, and 1930 to 1825 µg/mL for standard citric acid, canned beverage (7up, and Lipton ice tea), and bottled drinks (Fanta, and Sprite), respectively. Sunlight leads to decrease in citric acid concentrations from 1998 to 1848, and 1999 to 1451 µg/mL for canned beverage and bottled drinks, respectively due to the degradation of the acid.

KEY WORDS: Citric acid, beverages, storage effect, temperature effect, sunlight effect, spectrophotometry
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1. INTRODUCTION:

Monohydrate or anhydrous citric acid (CA), the most popular used acidulant to provide a sour taste to beverages and foods, which is also serves as a chelating agent, antioxidant, pH buffer, and preservative (Smith and Hong-Shum, 2011). Its E330 designation denotes it as a European food additive number. In 2020, China shipped almost 2.0 million tons of citric acid, making it the world's largest producer (Amato et al., 2020). Citrus fruits naturally contain this weak organic acid, which gives the fruit an acidic flavor and a sour taste. Additionally, it is a crucial intermediary in the Krebs cycle and the tricarboxylic acid cycle, which are both crucial processes in the metabolism of most living organisms. In addition to preventing kidney stones and being healthy for the skin, citric acid can aid the body fight oxidation (Singh et al., 2022). Citric acid comes in two different forms, monohydrate and anhydrous, as seen in Figure 1. If the temperature rises over 37°C, the monohydrate might become anhydrous.

*** Corresponding Author:**
Srwa Ramadhan Hamza
E-mail: Srwa.hamza@su.edu.krd
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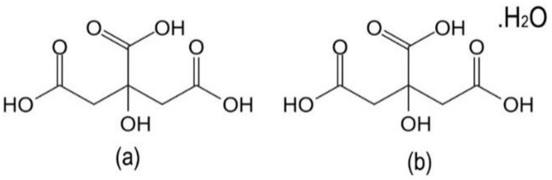


Figure 1: Chemical structure of anhydrous citric acid (a) and monohydrate (b).

Commercial production of citric acid involves microbial fermentation of a corn-derived sucrose or glucose carbohydrate substrate. Usually produced by feeding a substrate to black mold. *Aspergillus Niger* is the main microorganism to produce or synthesis of citric acid. The following three production methods have received Food and Drug Administration (FDA) approval: mycological fermentation utilizing Candida spp., plant sources (such as pineapple or lemon juice), and *Aspergillus Niger* fermentation liquor (Baker and Grant, 2018). According to 2018 research in Toxicology Reports, *Aspergillus Niger* has been used to produce around 99.0% of the citric acid produced worldwide since 1919. Citric acid is present in abundance in citrus fruits. Bananas, blueberries, grapefruits, apples, peaches, strawberries, tomatoes, oranges, pineapple, lime, and lemon are examples of fruits that contain citric acid. Citric acid is found in abundance in lime and lemon juice. Ready-to-consume lemon juice has a concentration of around 41.0 mg/kg, whereas about 31.0 mg/kg in concentrates lemon juice. In lime juice, the values are 39.0 mg/kg and 30.0 mg/kg (Lim, 2012).

There are several techniques that have been described for citric acid determination in foods, medicines, and beverages. These methods include spectrophotometry (Hartmann and Hillig, 1928, Pro and Nelson, 1956, Vasilikiotis et al., 1983, Indyk and Kurmann, 1987, Krug and Kellner, 1994, Themelis and Tzanavaras, 2001, Zarei et al., 2007), colorimetry (Junge, 1987, Zhou et al., 2022), amperometry (Matsumoto and Tsukatani, 1996), potentiometry (Godinho et al., 1988), enzymatic method (Henniger and Mascaro Jr, 1985), liquid chromatography (Vazquez Oderiz et al., 1994, Shojaee AliAbadi et al., 2022), chemiluminescence (Pérez-Ruiz et al., 1995, Zhike et al., 1998), and high-pressure liquid chromatography (Cunha et al., 2001, Scherer et al., 2012, Hijazi et al., 2022). Because of its intrinsic simplicity, inexpensive, and widespread availability in quality control laboratories, spectrophotometric method is perhaps the most practical analytical technique for routine examination (Jabbar and Faizullah, 2013, Jabbar and Faizullah, 2015). Beverages are exposed to different conditions in terms of temperature and the effect of light (sunlight) which leads to different chemical and physical changes. Where these factors affect the beverages as a result of the wrong storage in large warehouses and markets.

The aim of the present method is development of a sensitive, low-cost, fast, and simple spectrophotometric approach for citric acid determination in beverages. The approach is based on the citrate's complexation ability with the ferrate ion. Because citric acid forms a stable complex with Fe(III), the red-colored $[Fe(CN)_2]^+$ utilized as a reagent is destroyed upon contact with the sample (Krug and Kellner, 1994). The change in absorbance is recorded at 477 nm. The effect of different storage conditions on the citric acid concentration was studied. These conditions include time, temperature, and sunlight effect.

2. MATERIALS AND METHODS

2.1. Instrumentation

The spectrophotometer used was a JENWAY 6405 UV/Visible spectrophotometer with a 1.0 cm glass cell. The temperature of the solutions and the samples were set as needed for tests using a handmade heated water bath. The pH measurements were conducted using an Orion EA940 pH-meter that calibrated before using, with absolute accuracy limitations for the pH readings being specified by NIST buffers.

2.2. Reagents

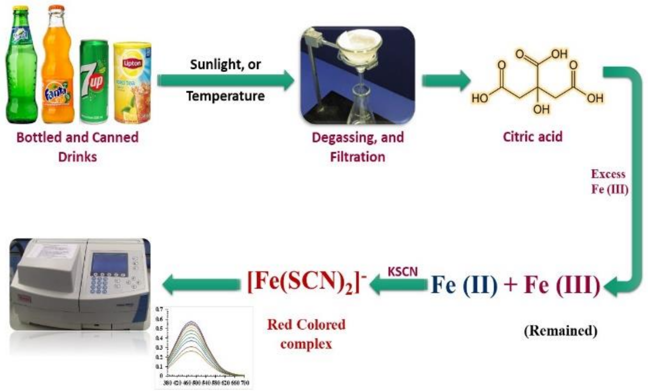
All chemicals were of analytical-reagent grade and all the solutions were made up with de-ionized water. The reagents suppliers were: citric acid trisodium salt and citric acid from Aldrich (Steinheim, Germany), KCl (potassium chloride) from Merck (Darmstadt, Germany), $FeCl_3$ (ferric chloride) and sodium thiocyanate (NaSCN) from Fluka (Buchs, Switzerland).

In order to prevent the ions from hydrolyzing, 0.05 mol/L HCl was added to the standard stock solution of $FeCl_3$, which had a concentration of 1000 mg/L. The required quantity of citric acid monohydrate (0.105 gm) ($C_6H_8O_7.H_2O$, molecular weight 210.14 g/mol) was dissolved in 1000 mL of deionized water to prepare a standard stock solution with 0.5 mmol/L of citric acid. Before to use, working solutions of citric acid and Fe(III) were prepared using the proper dilution.

2.3. Determination of citric acid

Aliquots of solutions with different concentrations of (0.50–50 mg/L) citric acid were transferred into a series of 10.0 mL calibrated volumetric flasks. Then, 1.5 mL, 10 mM of Fe solution was added. The pH of the solutions was adjusted to 1.5 at room temperature (25 ℃). After 2.0 minutes, a 1.0 mL volume of 600 mM thiocyanate solution was added. After completely mixing, the solution was left to stand for 1.0 minutes. The absorbance was measured at the maximum wavelength (477 nm). A blank experiment was conducted using the identical steps but without the citric acid solution.

Soft drink and beverage samples (three samples from each type: 7up, Sprite, Lipton ice tea, and Fanta) where purchased from Erbil local markets and stored in laboratory according to the studies (effect of temperature, and sunlight). For the canned and bottled beverages, the sole sample preparation steps were degassing, dilution, and filtering when necessary, but for the soft drinks, the appropriate volumes of the samples were diluted in de-ionized water. Three replicates were used for each treatment. Scheme 1 illustrates steps of citric acid determination.



Scheme 1: Illustration of citric acid determination steps.

3. RESULTS AND DISCUSSION

The reaction of ferrate ion and thiocyanate in an acidic medium yields a red color due to the production of $[Fe(SCN)_2]^+$ complex (Reaction 1), which has a maximum absorbance at 477 nm (Patel and Singh Patel, 1999, Silva Júnior et al., 2010). The complex color intensity decreases in the presence of citric acid, this was employed as a useful approach for the CA determination in canned juices. The experimental factors impacting the color development and its stability such as effect of pH, Fe(III) and thiocyanate concentrations, reaction time and temperature, as well as the spectrophotometric characteristics of the colored product, were thoroughly examined and optimized. These elements were changed independently while the rest remained constant. The effect of each variable was studied by using the arrangements as described under the procedure.

The absorption spectrum of a colored product shows a maximum absorption band at a wavelength of 477 nm for the formed colored complex of [Fe(SCN)₂]+. The addition of citric acid destroyed the former red complex between Fe (III) and thiocyanate ions (Figure 2).

$$[Fe(SCN)_2]^+ + 2\,(C_6H_5O_7)^{3-} \rightleftharpoons [Fe(C_6H_5O_7)_2]^{3-} + 2\,SCN^- \qquad (1)$$

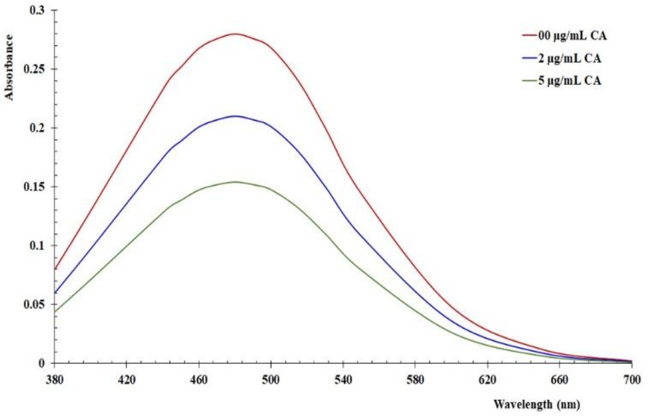


Figure 2: Absorption spectra of the colored complex and effect of 2.0 and 5.0 mg/L citric acid on the absorbance.

3.1. Optimization of experimental factors

The influence of pH on the reaction rate was studied in the range of 1.0 to 3.5 using KCl/HCl as a buffer solution. It was found that increasing pH would increase the absorbance of the reaction product as well as the absorbance up to pH 1.5, after which further increase in the solution pH resulted in a slight decrease in the absorbance of the reaction product, thus, 1.5 was found to be the most suitable pH for maximum absorbance (Figure 3a).

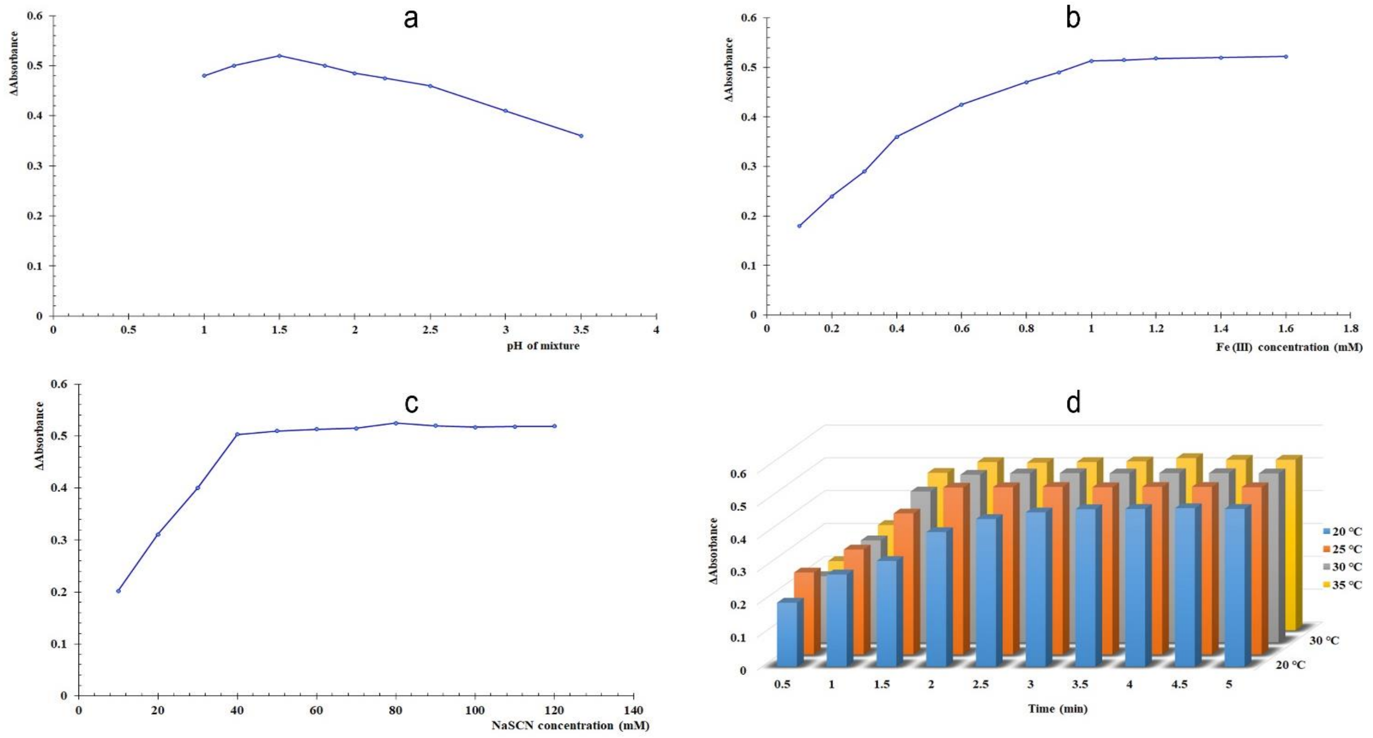


Figure 3: Effect of (a) pH, (b) ferrate ion concentration, (c) thiocyanate concentration, (d) reaction time and temperature on the reaction.

Fe (III) ion concentration effect on the formed color was also studied using 0.2–3.0 mL of 0.5 mM ferric chloride. It was found that increasing the concentration of Fe (III) leads to an increase the absorbance of the reaction product up to 1.0 mM, after which a further increase in the concentration of Fe (III) resulted in a very slight increase in the absorbance of the reaction product, thus, 0.5 mM Fe (III) was found to be the most suitable concentration for maximum absorbance (Figure 3b).

The effects of thiocyanate reagent were studied by measuring the absorbance of solutions containing a fixed concentration of CA (20 mg/L) and varying amounts of the respective reagent. The maximum color intensity of the complex was achieved with 2.0 mL of 0.01 mM thiocyanate as a reagent solution for CA. Although a larger volume of the reagent had no pronounced effect on the formed complex absorbance (Figure 3c).

The effect of reaction time and temperature were important parameters of the experiment and were investigated in the range of 0.5-5.0 minutes and 20-35 ℃ under the optimized chemical conditions. The absorbance of the colored complex gradually increased up to 2.0 minutes and then remained constant. In addition, 25℃ gets higher absorbance and raising the temperature to 35℃ doesn't effect on the absorbance (Figure 3d).

The absorbance remains stable for at least 48 hours. Thus, 2.0 min was selected as the optimal reaction time and 25 ℃ was select as the optimal temperature for the experiment and were sufficient for completion of the complexation reaction.

Table 1 summarizes the optimum chemical and physical variables for citric acid determination using the proposed approach.

Table 1: Optimal chemical and physical variables for citric acid determination using the proposed approach.

Variable	Studied range	Optimum value
pH	1.0 – 3.5	1.5
Fe (III) concentration	0.1 – 1.6 mM	1.0 mM
SCN^- concentration	10 – 120 mM	40 mM
Reaction time	0.5 – 5.0 min	2.0 min
Reaction temperature	20 – 35 ℃	25 ℃
Maximum wavelength	380 – 700 nm	477 nm

3.2. Calibration

Once the optimum reaction conditions were found, a set of standard citric acid solutions was prepared and analyzed in order to test the usefulness of the proposed procedure. As we can see (Figure 4), the linear calibration graph was ranged from 1.0 to 50.0 mg/L with a detection limit of 2.764 mg/L, a quantification limit of

8.376 mg/L, and a relative standard deviation of 0.87% (n= 12).

The recovery rates of pure citric acid at three concentration levels encompassing the high, medium, and low ranges of the calibration graph were used to determine the method's accuracy. Standard deviation (SD) as precision and recovery percentage (R%) as the accuracy of the proposed approach was calculated. As shown in Table 2, the accuracy findings demonstrated that the suggested techniques had high repeatability and reproducibility.

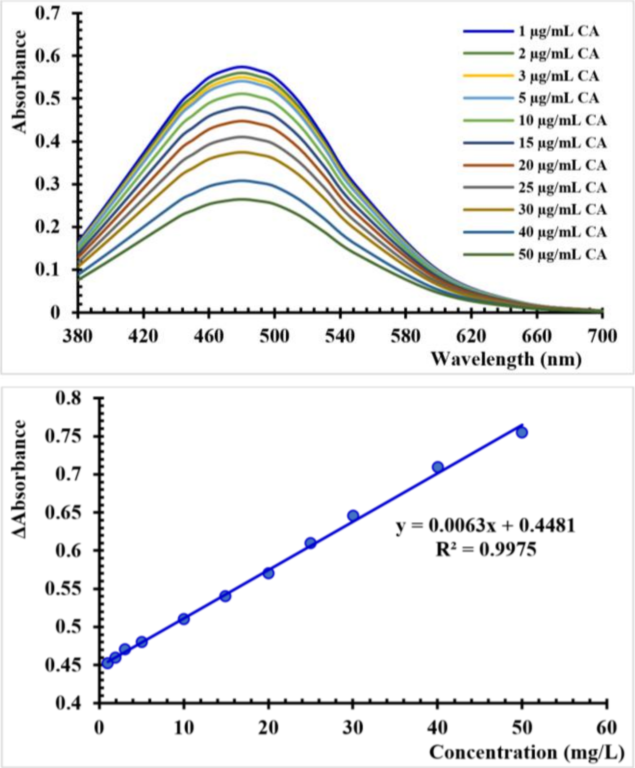


Figure 4: Calibration graph for spectrophotometric determination of citric acid.

Table 2. Evaluation of the precision and accuracy of the proposed approach.

Citric acid concentration (mg/L)		Recovery% ± SD
Added	Found ^{a}	
10.00	9.90	99.00 ± 0.87
25.00	24.79	99.16 ± 0.41
40.00	40.17	100.43 ± 0.19

^{a} The average value of five determinations.

3.3. Interference studies

The given approach may generally be used to determine a wide range of chemical compounds that form more stable complexes with Fe(III) ions than complexes of thiocyanate. It is appropriate for detecting complexing agents like pyro- and orthophosphate, citrate, tartrate, oxalate, CyTPA, DTPA, NTA, and EDTA as well as for determining the overall amount of complexing substances. Naturally, other species must be missing or present at concentrations two or more orders of magnitude lower when only one of the aforementioned species is to be detected (Krug and Kellner, 1994).

In general, drinks include two primary classes of potentially interfering chemicals. Different organic acid anions (tartaric, EDTA, phosphoric, lactic, and malic acid) that are the first group of interference compounds, compete with citrate ions in the selective union with metallic ions (such as the Fe(III)) that is in the primary reaction of the suggested approach. Aspartame, caffeine, sucrose, fructose, and glucose which are frequently present in the samples under analysis, are among the second category of possible interferences that might cause negative peaks in the signals.

The interference impact of various substances on the measurement of CA was investigated using the suggested spectrophotometric approach and under the optimum reaction conditions mentioned above. The average absorbance signal measured at a concentration of 20 mg/L of CA was used to establish the criterion for interferences as recovery per cent. The outcomes are shown in Figure 5.

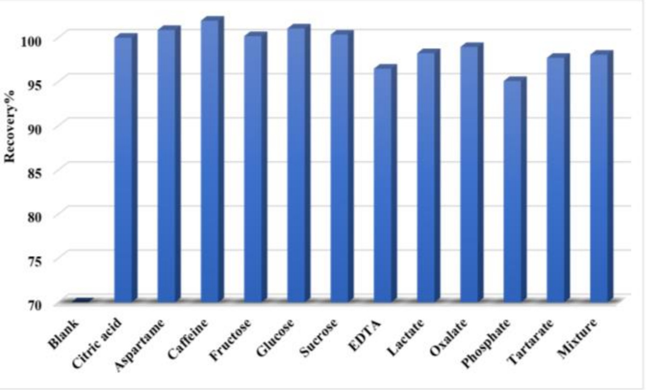


Figure 5: Schematic study of interferences. Comparison of 20 mg/L citric acid absorbance with 2.0 mg/L of organic acids and 400 mg/L other interfering agents.

As shown in the results, the suggested approach appears to be sufficiently selective, even at the most frequently occurring organic acids in the samples, such as tartaric, malic, lactic, phosphoric, and EDTA without the need for any pretreatment steps involving the potentially interfering substances elimination because the concentration of these species is about tenfold less than citric acid as labelled. When the mole ratio of interfered specie/citric acid is more than 1:10, the standard addition method is used to increase citric acid concentration and decrease interfered substance effect. The following interfering substances were studied in addition to those of anions of organic acids: glucose, lactose, fructose, aspartame, and caffeine. These compounds do not produce significant errors even at a mole ratio of 20:1 (substance/citric acid).

A mixture of compounds with a concentration of 2.0 mg/L per organic acids and 400 mg/L for other compounds with 20 mg/L citric acid was applied to this method and displayed in Figure 5. The findings of this study demonstrate that these substances have no significant effects on the method, allowing it to be referred to be a selective approach for citric acid determination in beverages with a recovery value ranged from 95.09 to 101.93 %. Therefore, the suggested approach cab be selectively applied for the determination of CA.

3.4. Citric acid determination in beverages

The suggested method for citric acid determination was successfully used to analyze commercial drinks available in local markets of Erbil City/Iraq. The suggested spectrophotometric approach can only be used on known matrix samples and only one dominant complexing agent because of interference effects. To avoid this problem, the concentration of citric acid was found by adding a standard citric acid solution to the beverage samples and making its concentration high in order to minimize the effect of the interfering substances and then subtracting the concentration of the added acid.

By analyzing different drinks that contain citric acid, including 7up, Sprite, Lipton ice tea, and Fanta, the established approach was tested. The results of the citric acid concentration in each sample are listed in Table 3. The resulted citric acid concentrations were compared to those obtained using the Boehringer-Mannheim enzymatic test kit method. The obtained errors for five replicate analyses for each sample were between -0.511 and 1.856%. The obtained results indicate that, on average, this method produces somewhat higher results than the enzymatic method. The higher values of the proposed method are explained by other organic acids positive interference that exhibit complexation capability with Fe (III) ions. Due to significant positive interference from phosphoric acid, Coca-Cola, Pepsi, and its Light products could not be evaluated for CA.

In the presented method it was possible to analyze different beverages, some drinks' turbidity and color caused inaccurate findings. This source of inaccuracy might be addressed by selecting an appropriate dilution for each product, often from the range of five to tenfold.

Table 3: Results were found for citric acid determination in beverages using the suggested approach and standard reference method.

Sample	Citric acid concentration (mg/L)		Error%
	Proposed method [a]	Reference method [b]	
Fanta lemon	3512.24	3485.54	0.766
Fanta orange	3305.09	3259.18	1.409
Lipton ice tea lemon	1728.31	1701.27	1.589
Lipton ice tea peach	2187.19	2147.34	1.856
Seven up	1928.22	1934.02	-0.300
Sprite	1579.94	1588.06	-0.511

[a] Mean of five replications.
[b] UV-Spectroscopy (Boehringer–Mannheim method).

3.5. Effect of storage

One of the most important objectives of this research is to find the effect of storage on the percentage of citric acid in beverages. As these beverages are exposed to different conditions in terms of temperature and the effect of light (sunlight) on changing the percentage of citric acid in the beverages. Where these factors affect the beverages as a result of the wrong storage in large warehouses and markets. This can happen as a result of storing beverages in places exposed to direct sunlight, which in turn leads to physical and chemical changes in these beverages. Especially that the temperatures in some warehouses reach 50 °C or more at summer due to the lack of proper ventilation and cooling for the storage conditions. Some of these beverages contain colored compounds that may be degraded with the effect of temperature, ultraviolet, and sun light leads to decrease the quality of the products.

To conduct this study, standard citric acid solution, canned 7Up, and bottle (glass) 7Up beverage samples were taken and divided into two groups. Where the first group was exposed to different temperatures, and the second group was exposed to direct sunlight, and then the acid concentration was found at specific times.

3.5.1. Storage temperature effect

The first group of solutions were stored at different temperatures in a dark place where the solutions were not exposed to light during storage. The effect of storage temperature at 10, 25, 35, and 45 ℃ was studied over a period of four months. The citric acid concentration was calculated on the first day of storage, and after each month for four months (Figure 6).

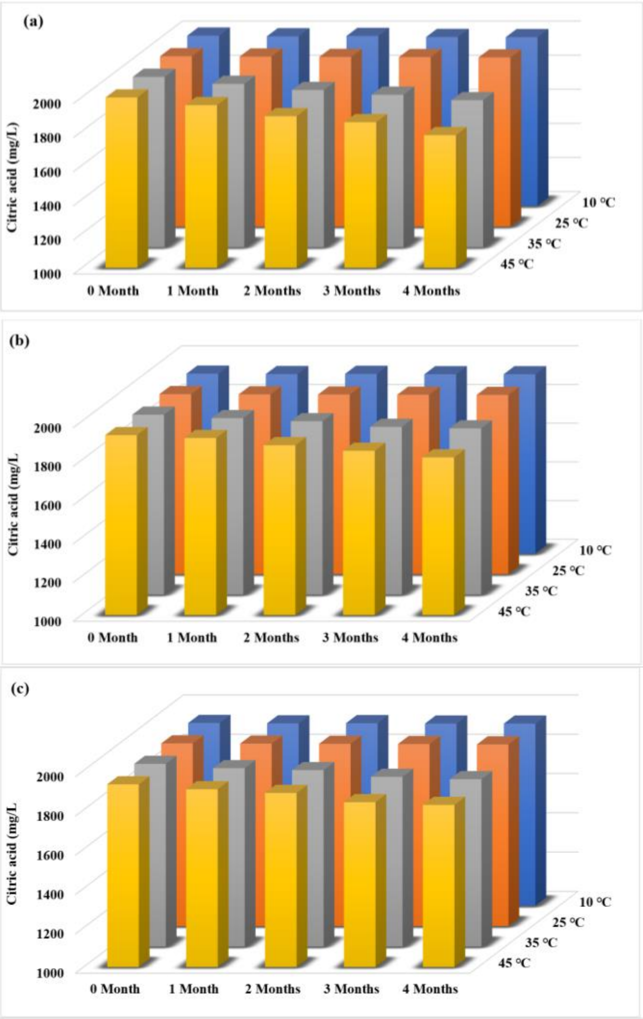


Figure 6: Effect of storage temperature on the (a) standard citric acid solution, (b) canned beverage (7up, and Lipton ice tea) citric acid, (c) bottled beverage (Fanta, and Sprite) citric acid.

Figure 6 a, b, and c shows the effect of storage temperature and storage period on the concentration of citric acid. Where we note that the concentration of citric acid in the standard solution, canned beverage, and bottle beverage is almost constant and does not change except at a very small rate at low temperatures (10 ℃) and room temperatures (25 ℃) for four months. While the acid concentration decreases with an increase in temperature from 35 to 45 ℃ with an increase in the storage period (Lisińska and Aniołowski, 1990) due to the degradation of citric acid (Quici et al., 2007). We also note in the figure that the effect of heat is almost the same for standard solutions, canned beverage, and bottled beverage, as the reason for this is that the storage conditions are in a dark place.

3.5.2. Effect of sunlight

Since beverages are stored in some stores in open places exposed to sunlight, or in some stores beverages are placed in places near glass windows exposed to direct sunlight. And there are other stores that put these drinks in refrigerators to cool them, but these refrigerators are in places exposed to the sun. Therefore, the second group of citric acid solutions were exposed to sunlight for specific periods during the months of May, June, July and August. Then, the concentration of citric acid in each case was determined.

Figure 7 shows the effect of sunlight on the concentration of CA. We note that the decrease in acid concentration in the standard solution and the canned beverage is slower than it is in the bottle beverages. As the sun's lights will directly affect the components of the bottled beverage. In addition to high temperatures, sunlight and ultraviolet rays may lead to the degradation of citric acid and the formation of chemicals harmful to human health (Quici et al., 2007).

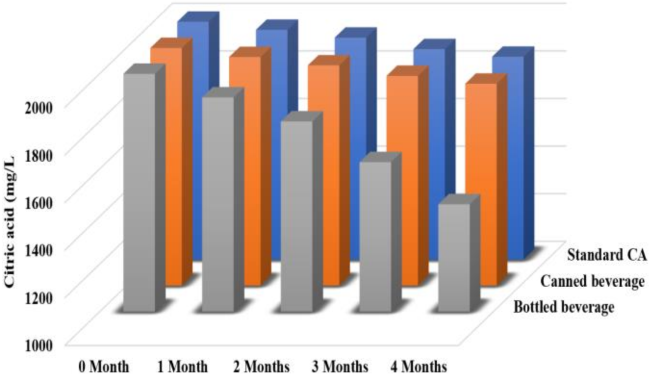


Figure 7: Effect of sunlight on the citric acid content.

We note in the figure that the decrease in acid concentration is faster in the months of July and August, and this may be due to the strength of the sun's light and the high temperatures in these months that help to disintegrate the acid. In general, exposure of stored beverages to sunlight with heat leads to a decrease in the concentration of citric acid, greater than the results of exposing beverages to high temperatures only.

4. CONCLUSIONS

The suggested spectrophotometric approach for citric acid determination in beverages satisfies the key requirements of routine analysis. It offers high sampling rates, minimum sample pre-treatment, inexpensive operational costs compared to enzymatic methods, and a wide range of CA concentrations for the direct detection. In addition, it exhibits high precision and accuracy across the board as well as appropriate selectivity. In addition to simplicity and affordability, the method's sensitivity is sufficient in terms of the concentration of citric acid in real samples. These characteristics suggest that this approach may offer advantages over pricey and time-consuming chromatographic methods that use enzymes to quickly and accurately measure the citric acid content in beverages. The pre-treatments include dilution when the concentration of the interfering compounds is too high, centrifugation to remove suspended particulates, and treatment with ultrasonic energy to remove the carbonic anhydride from carbonated beverages was used. In addition, from study of the effect of storing of drinks at various temperatures and exposing them to sunlight for varying period concluded that citric acid degraded into potentially harmful compounds and the percentages of degradation products increased with increasing of temperature and period of exposure to sunlight.

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Conflict of interest

The author declare no competing interests.

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