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QUANTITATIVE ANALYSIS OF LEMON JUICE

BY

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Abstract. Citrus fruits are the most widespread and consumed fruits in the world, being cultivated in more than 80 countries. Lemon juice can be used to clean grease or stains from various surfaces, as an air freshener, as a disinfectant, to remove dark sport and cellulite from skin. Lemon is considered a natural diuretic, control fat accumulation and improve enzymes actions. Vitamin C protects biologically active compounds from oxidative degradation; stimulates the biosynthesis processes in cells. Citric acid, mainly found in various fruits and vegetables, is extremely important in some enzymatic processes. The lemon juice fresh squired was analysed by the means of quantitative measurements of vitamin C using both iodometric and spectrophotometric methods, also the total acidity by titration with NaOH and the electrical conductivity were measured.

Keywords: lemon juice, iodometric and spectrophotometric quantitative analysis of Vitamin C, total acidity, electrical conductivity.

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1. Introduction

Citrus fruits are the most widespread and consumed fruits in the world, being cultivated in more than 80 countries. Brazil is the leader in citrus production, registering over 18.90 million tons of fruit in 2004, followed by the United States and China (Sawamura, 2016).

The World Citrus Organisation (WCO) forecasted that citrus production in the Northern Hemisphere in 2023 will be about 28.98 tons. That's up 12.21% compared to the prior year and 1.48% higher than the four-year average. Oranges are forecast to account for 50% of Northern Hemisphere production; followed by soft citrus, as: mandarins (29%); lemons (18%) and grapefruits (3%) (https://citrusindustry.net/2023).

Lemon is a fruit of the family *Rutaceae*, genus *Citrus*, species *Citrus limon*. It is one of the most well-known fruits in the citrus class, due to its multiple uses. The first lemons were planted in America in 1493 by Christopher Columbus, and currently there are lemon crops in countries such as the USA, Italy, Turkey, Israel, Spain and Greece. The etymology of the word "lemon" comes from the old French "limon".

Lemon juice can be used to clean grease or stains from various surfaces, as an air freshener, as a disinfectant, but not only that. Lemon peel oil can also be used to clean wooden surfaces or as a non-toxic insecticide (Klimek-Szczykutowicz *et al*., 2020).

In teaching experiments, lemon juice is sometimes used instead of an acid or as an invisible ink that develop colour in contact with a heat source.

In 2008, a study was conducted that aimed to evaluate the effect of polyphenols from lemon peel on the incidence of obesity in the case of a high-fat diet; Regulation of metabolism during the respective diet (Fukuchi *et al*., 2008).

The study was performed on 12 mice divided into 3 groups. They were fed for 12 weeks as follows (Fukuchi *et al*., 2008):

- Group I: Low-fat diet;
- Group II: Diet rich in fats;
- Group III: high-fat diet to which 0.5% polyphenols extracted from lemon peel were added.

The results of the experiment showed that the mice in the III group showed significant reductions in gaining weight, growth of adipose tissue, development of insulin resistance (Fukuchi *et al*., 2008).

Lemon extract is well known as a natural antioxidant, recently been discovered that it also helps to inhibit the appearance of cancer cells in the body. The lemon essential oil contains terpenoids that are well known for their pharmaceutical and physiological effects such as: antimicrobial effect or chemopreventive properties. The high content of flavonoids also contributes to the reduction of fat accumulations on the walls of the arteries (Klimek-Szczykutowicz *et al*., 2020).

Lemon is considered a natural diuretic, so it favours the elimination of toxins from the body. Having a composition similar to that of saliva, lemon juice stimulates the production of gastric juice in the stomach, which facilitates digestion (Klimek-Szczykutowicz *et al*., 2020).

The high amount of citric acid in lemon helps to maximize the activity of enzymes, thus improving the activity of the liver and also brings a considerable supply of vitamin C to the body. Lemon also has a rich fibres content, especially pectin.

Due to its astringent action, lemon is one of the best natural ingredients that are able to stop the excessive secretion of sebum, responsible for acne. Its use helps to deep clean the pores and eliminate the bacteria that aggravate this condition (Klimek-Szczykutowicz *et al*., 2020).

The acids and essential oils in lemon juice act as depigmenting agents for skin spots, being able to reduce the uneven coloration of skin spots. It is also recommended to be used in the case of age spots, or those caused by the sun (Klimek-Szczykutowicz *et al*., 2020; Zhu *et al*., 2023).

Among the benefits that lemon has on the skin is the ability to reduce cellulite. Frequent use improves circulation, minimizing the appearance of orange peel of skin. Due to the high content of vitamin C, lemon, in combination with coconut oil, can visibly reduce scars or stretch marks (Klimek-Szczykutowicz *et al*., 2020).

In the human body, vitamin C plays very important and complex roles, including:

• Protects biologically active compounds from oxidative degradation;

• Stimulates the biosynthesis processes of collagen, steroid hormones and some neurotransmitters;

• Participate in the synthesis of dopamine, adrenaline and noradrenaline in the nervous system and adrenal glands;

• Favours the synthesis of carnitine, which is important in energy transfer in cell mitochondria.

The lack of vitamin C in the body leads to the appearance of a form of avitaminosis called *scurvy* or *Barlow's disease* (inflammatory scurvy). This deficiency occurs in the case of insufficient dietary intake or digestive malabsorption and generally occurs in the elderly, alcoholics or patients fed by the means of infusions not supplemented with vitamin C.

Excess vitamin C in the body leads to agitation and insomnia, but it is not harmful because the excess is eliminated through urine and faeces. The recommended daily dose for adults is 60 mg of vitamin C, but even 10 mg is sufficient to avoid avitaminosis. Following a study, it was discovered that the LD50 for rats is 11900 mg vitamin C/kg body (Barrett, 1995).

2. Chemical Composition of Lemon

Lemon is used for both culinary and non-culinary purposes, primarily for juice, although the pulp and peel are also used, particularly in cooking. Lemon juice contains about 5-6% (about 0.3 M) citric acid and has a pH = 2-3. The distinctive sour taste of lemon juice makes it a key ingredient in many dishes around the world. On average, one lemon contains about 3 tablespoons of juice (50 mL) (Fakoor Janati *et al*., 2012).

The main compounds in essential oils extracted from lemons are terpenes that are part of the isoprenoid class, the most well-known being limonene (65.65%) - which gives the specific smell, γ-terpinene (9.01%), terpinolene, α-pinene and β-pinene (11%) (Fakoor Janati *et al*., 2012; Klimek-Szczykutowicz *et al*., 2020).

In lemon many chemical elements are to be found such as: Potassium: 138 mg; Calcium: 26 mg; Phosphorus: 16 mg; Magnesium: 8 mg; Sodium: 2 mg; Iron: 0.60 mg; Zinc: 0.06 mg; Copper: 0.037 mg (Fakoor Janati *et al*., 2012; Klimek-Szczykutowicz *et al*., 2020).

Among the vitamins, found in lemon are: Vitamin C: 53 mg; Vitamin B5: 0.190 mg; Vitamin B3: 0.100 mg; Vitamin B6: 0.080 mg; Vitamin B1: 0.040 mg; Vitamin B2: 0.020 mg; Vitamin A: 22 IU; Vitamin E: 0.15 µg (Fakoor Janati *et al*., 2012; Klimek-Szczykutowicz *et al*., 2020; Zhu *et al*., 2023).

Lemon extract also contain polyunsaturated fatty acids: 0.089 g, saturated fatty acids: 0.039 g; monounsaturated fatty acids: 0.011 g; cholesterol: 0 mg (Fakoor Janati *et al*., 2012; Klimek-Szczykutowicz *et al*., 2020; Zhu *et al*., 2023).

Ascorbic acid, also known as Vitamin C, is an organic acid with antioxidant properties that actively participates in the reduction and oxidation processes that take place in living cells thanks to the ascorbic acid - dehydroascorbic acid system that has the role of regulating the redox potential in cells, contributing to hydrogen transport. By slow oxidation, ascorbic acid turns into the dehydroascorbic acid (Moldovan *et al*., 2006) as is shown in Fig. 1.

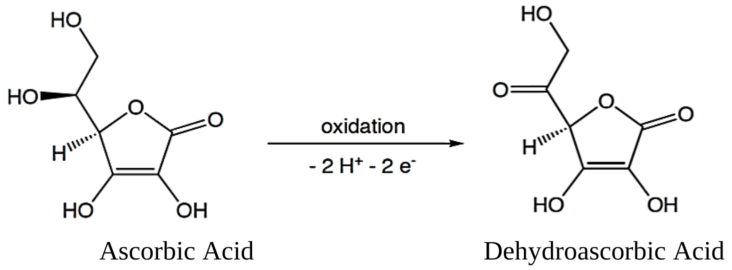

Fig. 1 − Ascorbic acid oxidation.

From a physical point of view, vitamin C forms colourless crystals, that are sensitive to heating and oxidation, which eliminates its biological effects.

Although the carboxyl group is not found in the structure of ascorbic acid, it still belongs to the class of acids. The acidic character is due to the enolic –OH group from the C3 carbon atom (Fig. 2), which is why ascorbic acid forms salts with dilute bases (Cernătescu and Cobzaru, 2014):

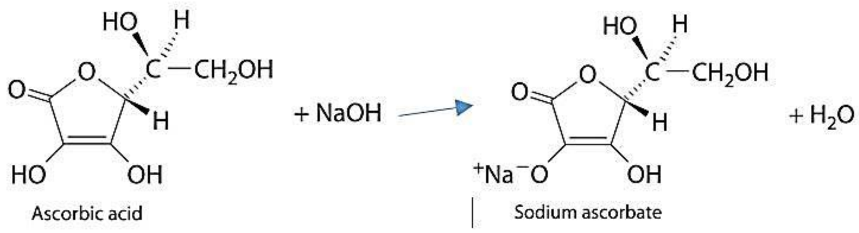

Fig. 2 – Ascorbic acid neutralization.

Vitamin C is stable in an acid pH, but very unstable in a neutral or basic pH and is easily dissociated in the presence of oxygen, heavy metal cations, light or oxidoreductases (ascobatoxidase, polyphenoloxidase). The vitamin C content of food is drastically reduced by the process of cooking or preserving the raw food material. Thus, the longer the storage period, the greater the loss of vitamin C (Cernătescu and Cobzaru, 2014; Rady *et al*., 2023).

3. Materials and methods

In experiments lemons from market have been used. Regents were from Merck and Sigma-Aldrich and were used without further purification.

For spectrophotometric analysis an LLG-uniSPEC4 LABWARE spectrophotometer has been used. The electrical conductivity and pH have been measured with an PeakTech® P 5307 device. All experiments were made in triplicate and the mean value has been used.

3.1. Determination of ascorbic acid in lemon

The dosage of vitamin C from lemon juice was done by the iodometric method namely: 10 mL of fruit juice, 30 mL of distilled water, 5 mL of KI, 5 mL of HCl and 1.5 mL of starch solution are introduced into an Erlenmeyer flask. The mixture is titrated with a KIO_3 solution, until the colour turns blue, that must persist for 30 seconds (Moldovan *et al*., 2006).

From a chemical point of view, the iodometric method is based on the oxidation of ascorbic acid with excess iodine, according to the reaction (Moldovan *et al*., 2006) as shown in Fig. 3:

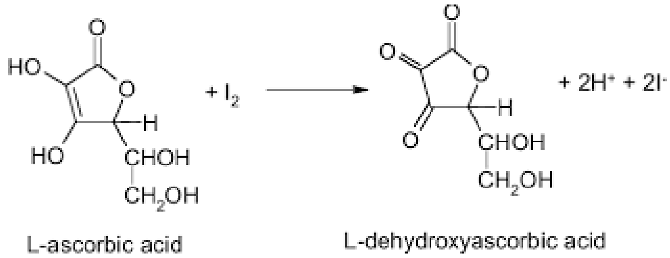

Fig. 3 – Ascorbic acid iodine identification method.

As an oxidant, iodine is generated *in situ* from potassium iodate and potassium iodide as shown below:

$$KIO_3 + 6HCl + 5KI \Rightarrow 6KCl + 3I_2 + 3H_2O$$

The number of moles of potassium iodate is calculated using formula (1), and the number of moles of oxidized ascorbic acid by titration is calculated using formula (2).

$$n_{KIO3} = \frac{V \cdot 0.0008}{1000} \quad (1)$$

$$n_{ac.\ ascorbic} = \frac{3 \cdot V \cdot 0.0008}{1000} \quad (2)$$

where: V – volume of KIO_3 used for titration, mL.

Since only 10 mL of the 50 mL sample were used in the titration, this value must be multiplied by 5.

Using the molecular mass of vitamin C (176 $g \cdot mol^{-1}$) in formula (3), the amount of vitamin C in the material under analysis is obtained by multiplication:

$$m = \frac{V \cdot 0.0008}{1000 \cdot 5 \cdot 176} \quad (3)$$

The vitamin C content per 100 g of plant material is calculated according to the following formula:

$$\%\ vitamin\ C = (V \cdot 0.0008 \cdot 5 \cdot 176)/(G \cdot 100) = (V \cdot 70.4)/G \quad (4)$$

where: V – volume of KIO_3 used for titration, mL;
G – the amount of material initially weighed, g;
V_{KIO3} = 0.1 mL.

3.2. Determination of ascorbic acid by spectrophotometry

The spectrophotometric method for determining ascorbic acid in aqueous solutions is based on its ability to decolorize potassium permanganate (530 nm) and dichromate (350 nm) (Fadhel, 2012).

A volume of 5 mL of $KMnO_4$ solution with a concentration of 40 ppm is introduced into 5 different flasks, and into each flask, 5 mL of ascorbic acid solution are introduced, with the following concentrations: 1, 4, 8, 12 and 16 ppm. The absorbance of the 5 samples is measured using a UV-VIS spectrophotometer at 530 nm (Fadhel, 2012).

3.3. Determination of total acidity in lemon juice

For lemons and oranges, the total acidity is expressed in grams of citric acid and the following ratio is taken into account that 1 mL of NaOH corresponds to 0.0064 dehydrated citric acid (Rady *et al*., 2023; Skoog and West, 2014).

In a 200-250 mL Erlenmeyer flask, 5 mL of lemon juice were added, that were diluted with distilled water (10 mL) to reduce its colour, 2-3 drops of phenolphthalein and the mixture was titrated with a 0.1N NaOH solution until a persistent pale pink colour developed. In the case of more coloured extracts, a blue litmus paper is used on which a drop of distilled water is applied and next to it drops of the titrated solution. The titration is considered finished when the drop in the beaker no longer colours the litmus paper red.
Total acidity is calculated using formula (5):

$$A_T\,(\%) = \frac{a \cdot F \cdot c \cdot 0.0064 \cdot 100}{G} \quad (5)$$

where: a – volume of NaOH 0.1N consumed in titration, mL;
c – dilution coefficient;
F –factor of 0.1 N NaOH solution;
G –volume of sample used, g.

3.4. Determination of electrical conductivity of lemon juice

Lemon juice is acidic and works as an electrolyte. The lemon itself acts as an electron reservoir that could transfer between the electrodes. When the two electrolytes (Cu and Zn) are immersed in lemon juice, their atomic structure breaks down, thus leading to the production of individual electrons. Oxidation occurs at the zinc electrode when it is immersed in lemon juice. Oxidation is followed by a release of electrons and advancement from the energy layers. Although the voltage produced by a lemon is very small, it can serve as an environment conductive to the production of energy. Thanks to this property, lemon can be used as a source of energy. Connecting four lemons in series can generate enough energy to light an LED (Rady *et al.*, 2023; Skoog and West, 2014).

The electrical conductivity of lemon juice can be determined using a conductometer. For the experimental determination of the electrical conductivity, 5 mL of lemon juice were measured, which were diluted with distilled water, then transferred into the conductometer tank and the electrode was immersed in the liquid to be analysed (Skoog and West, 2014).

4. Results and discussions

4.1. Determination of ascorbic acid in lemon

Quantitative analysis of vitamin C in food are based on its reducing properties. Ascorbic acid is oxidized under the action of oxidizing agents forming dehydroascorbic acid. The errors that may occur in the determination may be due either to the fact that the analysed foods also contain other substances that could be oxidized, or to the rapid oxidation of ascorbic acid, even during the preparation of the samples, which is why the dosing must be done very quickly.

According to the iodometric method (3.1), the amount of vitamin C in the material under analysis obtained by formula (3) is $9.09 \cdot 10^{-11}$ (g vitamin C/10 mL lemon juice), and the vitamin C content per 100 g of plant material calculated with formula (4) is 0.0704%. These results shows that the analyzed lemon juice fresh contains vitamin C.

4.2. Determination of ascorbic acid by spectrophotometry

The following values were obtained for the 5 potassium permanganate solutions of different concentrations, and the absorbance values are represented in the Fig. 4.

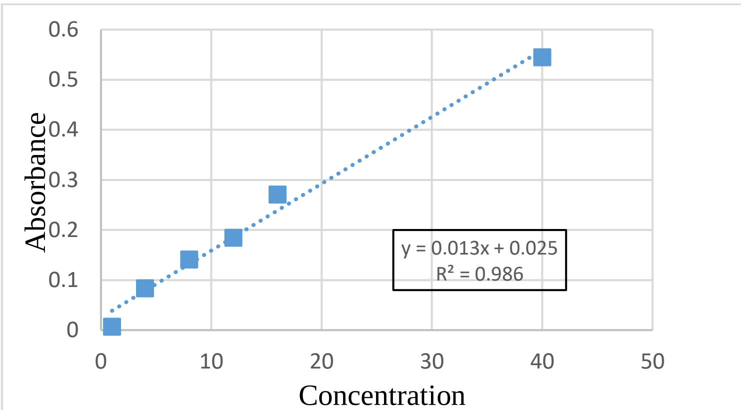

Fig. 4 – Calibration curve for $KMnO_4$.

Absorbance at λ =530 nm is 0.545 for the blank and 0.078 for the analysed sample.

According to the equation of the calibration curve, a concentration of 3.94 ppm ((0.078-0.0253)/0.0133) in the analysed sample was obtained. Since according to the specialized literature 1 mole of ascorbic acid is able to decompose 1 mole of $KMnO_4$, it means that the lemon juice under analysis has an ascorbic acid content of 36.06 ppm.

4.3. Determination of total acidity in lemon juice

Using experimental result in formula 1 and knowing that the volume of NaOH used in titration was V_{NaOH} = 52 mL, the total acidity is:

$$A_T\ (\%) = \frac{52 \cdot 1 \cdot 2 \cdot 0.0064 \cdot 100}{5} \Rightarrow A_T = 13.312\%$$

Following the experimental determinations using an electronic pH-meter, it was concluded that the analysed lemon juice has an acidic character indicated by the pH value of 2.6, a value that corresponds to the data from the literature (Fadhel, 2012).

4.4. Determination of electrical conductivity in lemon juice

The value displayed on the device's display was 4454 $mS\cdot cm^{-1}$ (445.2 $S\cdot m^{-1}$), which indicates that lemon juice has a very good electrical conductivity.

5. Conclusions

Citrus fruits are the most widespread and consumed fruits in the world, being cultivated in more than 80 countries, the worldwide production increasing each year. Lemon juice has many applications in food, housework, chemistry, biochemistry and biology. Lemon essential oil also has many active compounds with bioactive applications.

Quantitative analysis of vitamin C was done by the iodometric method, that is based on the oxidation of ascorbic acid with iodine in excess. From the analysis an amount of vitamin C = 0.0704% was recorded.

The spectrophotometric method for determining ascorbic acid in aqueous solutions is based on its ability to decolorize potassium permanganate (530 nm). After recording the calibration curve of $KMnO_4$ solution and the measurements of sample absorption at 350 nm, a concentration of 3.94 ppm $KMnO_4$ in the analysed sample was obtained, the correspond to an ascorbic acid content of 36.06 ppm.

The total acidity, expressed in grams of citric acid was measured by titration with NaOH, knowing that 1 mL of NaOH corresponds to 0.0064 dehydrated citric acid. From experimental result a total acidity of 13.312% was obtained, that correspond with literature data. The experimental determinations using an electronic pH-meter, it was concluded that the analysed lemon juice has an acidic character indicated by the pH value of 2.6.

The electrical conductivity of lemon juice has been determined using a conductometer. The value displayed on the device's display was 4454 $mS \cdot cm^{-1}$ (445.2 $S \cdot m^{-1}$), which indicates that lemon juice has a very good electrical conductivity.

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ANALIZE CANTITATIVE ALE SUCULUI DE LĂMÂIE

(Rezumat)

Citricele sunt cele mai consumate fructe în întreaga lume, fiind cultivate în mai mult de 80 de țări.

Sucul de lămâie poate fi utilizat pentru a îndepărta petele de grăsime de pe diverse suprafețe, ca aromatizant pentru încăperi, ca dezinfectant, pentru a îndepărta petele și celulita pielii. Lămâia este un diuretic natural, controlează acumularea țesutului adipos și îmbunătățește acțiunea enzimelor. Vitamina C protejează compușii biologic activi împotriva oxidării și stimulează o serie de procese de biosinteză la nivelul celulelor. Acidul citric, care se găsește în numeroase fructe și legume, este foarte important într-o serie de procese enzimatice.

Sucul de lămâie, proaspăt stors a fost supus unor analize cantitative pentru a determina conținutul de vitamină C, atât prin metoda iodometrică, cât și prin spectrofotometrie, aciditatea totală, prin titrare cu soluție de NaOH și s-a măsurat conductivitatea electrică.