FURTHER INSIGHTS TOWARD VITAMIN C DETERMINATION AND STABILITY. PROPOSAL OF A NEW QUANTIFICATION METHOD

AVALIAÇÕES ADICIONAIS DA DETERMINAÇÃO E ESTABILIDADE DA VITAMINA C. PROPOSTA DE UM NOVO MÉTODO DE QUANTIFICAÇÃO

Carlos Alberto VIEIRA[1], Vanessa Grazieli MILANI[2], Vanessa Carolyne Figueiredo de LISBÔA[2]; Camila Jacob Ferreira MENEZES[2]; Luma Chezira Rabatone JORGE[2];José Roberto Giglio[3]

1. Biologist, Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil; 2. Scientific Initiation students in the same Department; 3. Full Professor in Biochemistry in the same Department. jrgiglio@fmrp.usp.br

ABSTRACT: This work aimed at an evaluation of the classical iodine method for quantification of vitamin C (L-ascorbic acid) in fruit juices, as well as at a search into the stability of this so popular vitamin under different conditions of pH, temperature and light exposition, in addition to a proposal of a new quantification method. Our results point to the persistent reversibility of the blue color of the starch-triiodide complex at the end point when using the classical iodine titration, and the overestimation of the true vitamin concentration in fruit juices. A new quantification method is proposed in order to overcome this problem. Surprising conclusions were obtained regarding the controversial stability of L-ascorbic acid toward atmospheric oxygen, at low pH, even in fruit juice and at room temperature, showing that the major problem concerned with aging of fruit juices is proliferation of microorganisms rather than expontaneous oxidation of L-ascorbic acid.

KEYWORDS: Vitamin C. Oxidation-reduction. Stability.

INTRODUCTION

Vitamin C, also called L (+) ascorbic acid and antiscorbutic factor, is one of the most popular vitamins and vital for humans, primates, guinea pigs, some birds and fishes, all of them unable to synthesize it from glucose. Its oxidized form is named dehydroascorbic acid (Figure 1) (DEVLIN, 2006; NELSON ; COX, 2008).

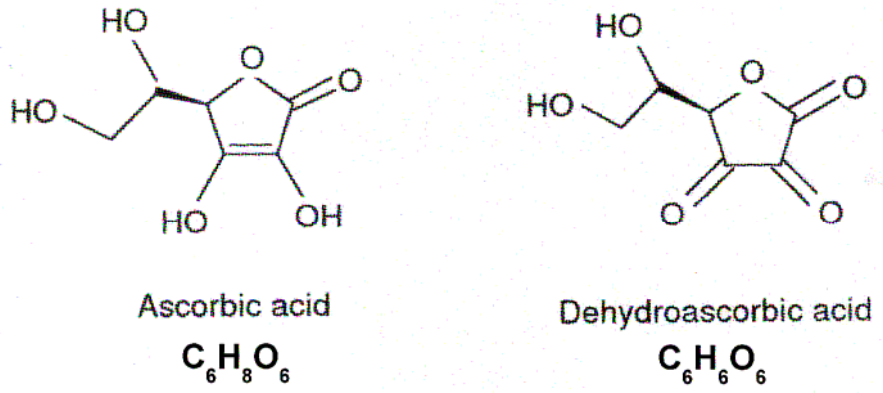

Figure 1. Structures of ascorbic and dehydroascorbic acids

"Vitamin" derives from the Latin word "vita" (life) + amine, an improper name introduced by Funk in 1911, when it was believed that all similar vital compounds were amines. This assumption was not later confirmed with the discovery of new non-nitogenated representants, such as vitamins C, A, D, E and K, the last four soon recognized as being fat-soluble, in contrast with about a dozen water-soluble vitamins, including the B complex and vitamin C. All the fat-soluble vitamins incorporate a high proportion of hydrocarbon structural elements. There are one or two oxygen atoms present, but the compounds as a whole are nonpolar. In contrast, a water-soluble

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vitamin contains a high proportion of the electronegative atoms oxygen and nitrogen, which can form hydrogen bonds to water; therefore, the molecule as a whole is water-soluble (HILL; KOLB, 1995). The name "vitamin" may not be apt today, but it is now too much widely accepted (English: vitamin; French: vitamine; German: vitamin; Portuguese, Spanish, Italian: vitamina; and even Japanese: bitamin) to be dislodged, even being derived from the original term "vitamine", whose unsuitability was tentatively attenuated by removal of the final "e" to give "vitamin".

Ascorbic acid (here abbreviated as AA) is essencially a reducing agent, able to convert Fe^{3+} to Fe^{2+} , as well as Cu^{2+} to Cu^{+}. This reducing activity is fundamental to keep iron ions in the ferrous state, as required by 4-prolyl and lysil hydroxylases to catalyse the formation of a consistent collagen, the fibrous protein which provides the structural framework for tissues and organs. Pro hydroxylation stabilizes collagen, whereas Lys hydroxylation provides sites for aldol interchain crosslinks and for glycosylation by specific glycosyltransferases of the endoplasmic reticulum. In the absence of AA, these bonds do not form, thus leading to fragility of capillaries and to the typical fractures of scurvy. AA also keeps reduced the iron ions of other enzymes envolved in the hydroxylation of dopamine to noradrenaline, whose lack is a cause of emotional lability. It is also required for the synthesis of carnitine which, combined with fatty acids, transports them for mitochondrial oxidation allowing muscles to use fatty acids as fuel, and whose lack leads to muscle weakness. All these symptoms manifest themselves in scurvy as a consequence of the absence or deficiency of AA. Manifestation of advanced scurvy includes also tooth loss, poor wound healing, bone pain and eventually heart failure, while milder cases are accompanied by fatigue, irritability and respiratory tract infections (DEVLIN, 2006; KAMOUN; LAVOINNE; VERNEUIL, 2006; NELSON; COX, 2008; PETEROFSKY, 1991).

The oxidation potential of the ascorbic-dehydroascorbic system (E_o = + 0.127 volts) is lower than those of the iodine-iodide (E_o = +0.540 volts) and of the ferric-ferrous (E_o = + 0.771 volts) systems (POSTMA; ROBERTS; HOLLENBERG, 2001). Reduction of Fe^{3+} to Fe^{2+} is also an important step for absorption of iron, whose major site is the duodenum. This reduction, however, occurs in the stomach, due to the low pH of gastric juice, and may be achieved by AA, making iron absorption easier because, in the reduced form, it is more easily dissociated from ligands. Some iron-containing compounds bind the metal so tightly that it is not, or at least, less available for assimilation. An example is spinach where some of its iron is bound to phytate, making this vegetable a poor nutritional source of iron (DEVLIN, 2006).

A previous paper dealing with this subject was published by Ciancaglini et al, (2001). Using starch as an indicator, when all the AA has been converted to dehydroascorbic, the additional I_2 reacts with I^- to form the linear triiodide ion I_3^- which combines with starch to form the deep blue starch-triiodide ion complex, thus signaling the end point of the titration (POSTMA; ROBERTS; HOLLENBERG, 2001).

In addition, using the I_2 method, experiments were carried out to evaluate the content of AA in ripe and unripe oranges, as well as in other ripe fruits, to search for probable interfering components, to investigate the influence of pH in the determination and stability of AA under different conditions, to verify the action of H_2O_2 on AA and to describe the use of Fe^{3+} replacing I_2 as titrant of AA, based on the observed difficulty to precisely detect the end point due to the continuous fading of the blue color of the I_3^-starch complex. Recently, a study proposed a method for analyzing AA by High Performance Liquid Chromatography using a hydrogen type ion exchange chromatogryaphy (ROSA et al, 2007). Addional articles dealing with quantification and stability of AA include: AUSTRIA; SEMENZATO; BETTERO, (1997), CARR; FREI, (1999), NAIDU (2003), ROIG; RIVERA; KENNEDY, (1993).

OBJECTIVES

The aim of the present article is an evaluation of the classical method for quantification of AA in fruit juices, based on its ability to reduce iodine to iodide, according to the following reaction:

$$\underset{\text{Ascorbic acid}}{C_6H_8O_6} + I_2 \rightarrow \underset{\text{Dehydroascorbic acid}}{C_6H_6O_6} + 2I^- + 2H^+$$

MATERIAL AND METHODS

All reagents used were of analytical grade and included: iodine (I_2) ≥ 99.8%; potassium iodide (KI) 99.99%; arsenic (III) oxide (As_2O_3) 99.995%; sodium hydroxide (NaOH) pellets ≥ 98%; sodium bicarbonate ($NaHCO_3$) ≥ 99.5%; soluble ACS starch; 37% hydrochoric acid ACS reagent; benzoic acid ≥ 99.5%; L-ascorbic acid ≥ 99%; D (-) fructose ≥ 99%); D (+) glucose ≥ 99%; hydrogen

peroxide solution, 29.0 – 32.0%; potassium ferricyanide [$K_3Fe(CN)_6$] 99.99%; iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) ≥ 98%; sodium thiocyanate (NaSCN) ≥ 99.99%; and 2,6-dichloroindophenol (Sigma-Aldrich).

Standardization of the aqueous iodine solution was carried out basically as previously reported (CIANCAGLIN et al , 2001) using a 0.1000 N (0.0250 M) freshly prepared primary standard solution of As_2O_3, final pH = 6.0.

Titration of the iodine solution

Ten (10.0) mL of the 0.1000 N solution of As_2O_3 were treated with 20 mL H_2O + 0.5 g of $NaHCO_3$ + 1 mL of the 1% starch solution (final pH = 8.1). The iodine solution was then dropped from a 25.0 mL burette up to the first light blue tone, indicative of the end point.

This standardized iodine solution was used, as reported below, to quantify the amount of AA in several fruit juices, under different conditions, as well as that of an aqueous solutions of pure AA as a function of time, in order to study its stability in contact with atmospheric oxygen.

$FeCl_3.6H_2O$ as a new titrant of AA

The proposal of a new method to evaluate the amount of AA in fruit juices, as justified in the Results and Discussion section, based on the ability of Fe^{3+} to oxidize AA to dehydroascorbic acid, requires a standard solution of Fe^{3+} to be used as substitute of I_2.

For that, we have used the above standardized solution of I_2 to titrate an aqueous solution of pure AA and this latter to titrate the $FeCl_3.6H_2O$ solution to be used for quantification of AA in fruit juices, using as an indicator the ion SCN^- which, in excess over the Fe^{3+} ions from the last drop of the titrant at the end point, forms the red complex $[Fe(SCN)_6]^{3-}$.

The overall sequencial steps for use of this solution as a titrant of AA is depicted below:

1) Standardization of the I_2 solution (A) with As_2O_3 as above reported.
2) Standardization of AA solution (B) with A.
3) Standardization of the $FeCl_3.6H_2O$ solution (C) with B.
4) Use of C to quantify ascorbic acid in fruit juices.

Fruit juices, as shown below, were obtained by direct squeezing (crude juice), or by filtration, ultrafiltration or centrifugation of the crude juice.

In order to make easier the understanding of the whole process, we depict below the reactions envolved:

1) $I_2 + I^- \rightarrow I_3^-$ (iodine solution)
2) $As_2O_3 + 2\ I_2 + 2\ H_2O \rightarrow As_2O_5 + 4\ I^- + 4\ H^+$
3) Starch + $I_3^- \rightarrow$ blue starch – I_3^- complex
4) $C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2\ I^- + 2\ H^+$
5) $C_6H_8O_6 + 2\ Fe^{3+} \rightarrow C_6H_6O_6 + 2\ Fe^{2+} + 2\ H^+$
6) $Fe^{3+} + 6\ SCN^- \rightarrow [Fe(SCN)_6]^{3-}$

Additional experiments, complementing this work, were also carried out and include: search into the hypothetic interference of β-carotene or fructose in the inconsistency of blue color of the starch – I_3^- complex for detection of the end point using the iodine method; AA content of ripe versus unripe fruit; microbiological examination of the juice to detect the presence of fungi and bacteria as a function of time; oxidation of the juice AA by H_2O_2, stability of pure AA in aqueous solution in contact with atmospheric O_2 at 4°C. Details of these experiments will be given in the Results and Discussion section.

RESULTS AND DISCUSSION

Standardization of the iodine solution with 0.1000 N As_2O_3:

The first standardized iodine solution was shown to be 0.0494 N.. The reaction $As_2O_3 + 2\ I_2 + 2H_2O$ to give $As_2O_5 + 4I^- + 4\ H^+$ is highly reversible. At pH values between 4 and 9, As_2O_3 can be titrated with I_2 but, in strongly acid medium, As_2O_5 is reduced to As_2O_3, the reaction therefore going from right to left (POSTMA; ROBERTS; HELLENBERG, 2001). This is why $NaHCO_3$ is added in the As_2O_3 solution, thus raising its pH to a final value of 8.1.

Determination of AA in ripe and unripe oranges (*Citrus sinensis*), expressed in mg/100 g

Two peeled ripe oranges, weighing 301.34 g, were liquefied in a blender and centrifuged at 6,950 x g for 10 min at room temperature, using a Sorvall RC2-B centrifuge. The residual pellet was stirred with 100 mL H_2O and recentrifuged as above. Total supernatant volume was 289 mL. An aliquot of 100.0 mL of the juice + 3 mL of 15% (v/v) HCl + 3 mL of the starch solution consumed 8.9 mL of the 0.0494 N I_2 solution (first end point). This and all other assays below were always run in duplicate or even triplicate when necessary, using then a mean value for calculation. Results are expressed as mean ± SD.

Calculation:

8.9 mL x 0.0494 mEq./mL = 0.440 mEq. of I_2 or AA.

0.440 x 88.06 = 38.72 mg of AA/100.0 mL of juice, or 111.89 mg/289 mL or 301.34 g of pilled orange = 37.13 mg/100.0 g.

The same procedure, using now unripe oranges, gave 34.25 mg/100.0 g. Therefore, ripe oranges showed to be about 8.4% richer in AA. Obviously this value may vary in function of the maturation level of the fruit, as well as of the fruit species. Note also that the "first" end point was considered, since we have observed that, for fruit juices, the turning point is sometimes dubious due to the inconsistency of the first blue color. This inconvenience persisted for all titrations of fruit juices with I_2, but it was attenuated by removal of solid debris through centrifugation, filtration and mainly ultrafiltration under N_2. A sharp end point is however achieved when pure AA solution is titrated.

The fruit juice titrations which follow were carried out with ripe fruit.

Lemon, pineapple, acerola and carambola

"Limão cravo"(*Citrus linonia*),a Brazilian lemon of the family Rutaceae; pineapple (*Ananas comosus*); acerola (*Malpighia emarginata),* an acidic cherry-like fruit very rich in AA; and carambola (*Averrhoa carambola),* star-fruit, were assayed.

Results were: 14.62 mg/100.0 g, 23.44 mg/100.0g, 883.74 mg/100.0g and 41.59 mg/100.0 g, respectively (Table 1). Note that the lemon is poorer in AA than the orange. Its higher acidity is due to its high content of citric acid (5 to 8 g/100.0 mL of juice). Acerola was the "champion" among all fruits we have so far analyzed. The data above show that 10 g of it supply all the AA which an adult needs for 24 h.

Table 1. Percentage % (m/m) of AA in some typical fruits.

Fruit	mg AA/100.0 g
Ripe orange	37.13 ± 0.05
Unripe orange	34.25 ± 0.04
Lemon	14.62 ± 0.01
Pineaple	23.44 ± 0.04
Acerola	883.74 ± 1.88
Carambola	41.59 ± 0.07

The simple procedure described above can be extended to any fruit able to be liquefied in a blender, even if some water is needed to help the extraction of AA. The final volume, after centrifugation, should then be considered for calculation.

A second approach is to replace centrifugation by filtration through a Whatmann number 1 filter paper. As stated above, a final refinement is the use of ultrafiltration under N_2, which may follow centrifugation or filtration, using an Amicon device with a membrane able to retain molecules with mol. weight > 1,000. The ultrafiltrated solution is now extremely clean, free of colloidal particles, but still coloured, thus indicating that at least β-carotene (mol. weight 536.85) is present. This clean solution was able to retain the blue color of the I_3^- starch complex for at least 1 h, while the material on the ultrafiltration membrane lost the blue color after 5 – 10 min.

The conclusion was that interfering substances in the fruit juice, with mol. weight > 1,000, react with I_2 within a relatively short time, making reversible the appearance of the blue color indicative of the end point. For a more accurate evaluation of AA in fruit juice, using the I_2 method, we suggest the following procedure steps: liquefaction in a blender → centrifugation or filtration → ultrafiltration.

β-carotene, which is a highly unsaturated compound, was initially considered as probably responsible for the inconsistency of the blue color, hypothetically due to an addition reaction of I_2 with a double bond. Its exclusion was however assumed after extraction of this pigment from tangerine (*Citrus reticulata*) juice (260 mL after centrifugation) with n-hexane (20 mL), followed by titration of the organic phase, yellow and turbid, + 30 mL H_2O + 0.5 mL of 15% HCl + 1 mL of 1% starch solution. The blue color soon appeared and persisted for at least 30 min. Fructose and glucose, usual reducing fruit sugars, were also excluded because they also did not react with I_2.

Therefore, high mol. weight (> 1,000) substances in the fruit juices are responsible for reversibility of the turning point in the I_2 method and they can be removed by ultrafiltration.

Influence of pH

Still concerned with the reversibility of the turning point, we investigated the influence of pH on this intriguing inconvenience.

Centrifuged tangerine juice was brought to pH values of 2 and 3 (with 5 M HCl), as well as 4, 5 and 6 (with 5 M NaOH). Titration with I_2 showed that the blue color was more persistent at pH values of 2 and 3, but at pHs 4 to 6 the color changed gradually to pink, fading rapidly.

Based on the equilibrium reaction:

$$I_2 + 2\ OH^- \rightleftharpoons I^- + IO^- + H_2O$$

it is clear that higher pH values favour conversion of I_2 to $I^- + IO^-$, thus fading the blue color of the I_3^- - starch complex.

A blank titration was then run with 50 mL H_2O + 0,5 mL of 15% HCl + 0,5 mL of 1% starch (final pH 2.26). The first two drops of I_2 produced the blue color which persisted up to pH 8 to 9, bleaching at higher pH values. The conclusion was that bleaching in the fruit juice titration is not due only to a decreased I_2 concentration at pH 6, but probably also to a consumption of IO^- by oxidation of some organic interferent, which is favoured at higher pHs. Therefore, pH 2 to 3 is recommended for titration of fruit juices by I_2.

Stability of AA

L-Ascorbic acid is an antioxidant, reacting enzymatically or nonenzymatically with reactive oxygen species which, in mammals, play an important role in aging and cancer (NELSON ; COX, 2008). Its reducing properties led to its postulated instability and belief that it is easely oxidized by atmospheric oxygen (BAYNES; DOMINICZAK, 2005; ; BROWN, 2000; CIANCAGLINI et al, 2001; SACKHEIM; LEHMAN, 1998; TIRAPEGUI, 2000). Even astonishing statements that frozen fruit pulps are completely devoid of vitamins are found (VINHOLIS, 2002).

In order to investigate the fall of AA concentration as a function of time and try to preserve it, we have undertaken the following experiments:

a) An aqueous solution of pure AA was prepared, containing about 5 mg/mL, pH = 3.0. Immediate titration (zero time) of 10.0 mL of this solution consumed 11.1 mL of a fresh 0.0515 N I_2 solution, what corresponds to 5.03 mg AA/mL.

This solution was kept at 4°C in an Erlenmeyer flask loosely covered with a beaker in the refrigerator. Daily titrations, along one week, showed practically the same results, with variations < 1%.

Part of the original solution was kept frozen and also did not show any difference after one week.

Finally, another aliquot was kept at 24°C in a stopped volumetric flask, exposed to day light. AA dropped to 4.72 mgAA/mL, 94.4% of the original value.

These results show that at 4°C, in the dark, AA is stable for at least one week, even in the presence of atmospheric air. A slight decrease of 5.6% occurred however at 24°C, after one week in the laboratory, with exposition to day light, even in a tightly stopped vessel (Table 2)

Table 2. Stability of pure AA in aqueous solution.

Time (days)	Conditions	mgAA/mL	% remaining
zero	Freshly prepared solution	5.03 ± 0.08	100.0
7	4°C, day light, loosely covered	5.00 ± 0.08	99.5
7	Frozem, dark, loosely covered	5.03 ± 0.07	100.0
7	24°C, day light, tightly stopped	4.72± 0.08	94.4

b) Similar experiments were carried out with freshly-prepared and diluted acerola juice that was centrifuged and filtered through glass wool.

The original pH = 3.4 was brought to 3.0 with 15% HCl. Titration of 10.0 mL of the clear solution consumed 4.95 mL of 0.0515 N I_2, therefore 22.45 mg/10.0 mL = 2.24 mgAA/mL (zero time).

Part of this solution was kept at 24°C in an Erlenmeyer flask as above (solution A), subject to day light. A second aliquot (B) was kept in the refrigerator at 4°C in the dark. Finally, a third. one (C) received 1 mg of benzoic acid/mL and was kept as A (Table 3).

Table 3. Stability of AA in acerola juice (loosely covered).

Time (days)	Conditions	mgAA/mL	% remaining
zero	Freshly prepared juice	2.24 ± 0.04	100.0

7	24°C, day light (A)	2.13 ± 0.04	94.9
7	4°C, dark (B)	2.24 ± 0.04	100.0
7	24°C, day light, benzoic acid (C)	2.24 ± 0.03	100.0
Addional			
4	24°C, day light (B)	2.13 ± 0.03	94.6
4	24°C, day light, benzoic acid (C)	1.63 ± 0.03	72.8
22	the same (C)	0.50 ± 0.01	22.3
34	the same (C)	0.16 ± 0.00	7.1
40	the same (C)	0.10 ± 0.01	4.5
42	the same (C)	0.08 ± 0.00	3.6

Titration of A after one week consumed 4.70 mL of the I_2 solution, therefore 21.3 mg/10.0 mL = 2.13 mgAA/mL = 94.9% of the original value. The original solution, now a suspension, was turbid and bad smelling. Microbiological analysis showed it was plenty of fungi and mainly bacteria, while B and C were limpid, free of microorganisms, and revealed the same concentration of AA as that of the original solution.

B and C were now left at room temperature and exposed to day light for 4 days more. Proliferation of bacteria was extremely high in B, but not even started in C. As it is known, benzoic acid is used as a preservative for foodstuffs to avoid spoilage due to growth of fungi and bacteria. To our surprise however, the AA concentration dropped to 2.13 mgAA/mL in B (due to a slow oxidation as expected) but to 1.63 mgAA/mL in C, 94.9% and 72.8%, respectively, of the original value. The higher decrease in C was probably due to partial esterification of AA by benzoic acid, since temperature and an excess of H^+ ions favour setting up of the equilibrium

$$R\text{ - }COOH + HOR' \rightleftharpoons R\text{ - }COOR' + H_2O$$

(BEYER; WALTER, 1996). Estimation of AA concentration in C was then followed up to 42 days and the results were: 0.50 mgAA/mL after 22 days; 0.16 mg/mL after 34 days; 0.10 mg/mL after 40 days; and 0.08 mg/mL after 42 days. No bacteria or fungi were detected. In addition, the juice was not bad smelling. A slight turbidity was identified as insoluble inert material .

Once again we see that, at 4°C and in the dark, AA is relatively stable over one week, even in the fruit juice. Decrease at 24°C was still modest (5.11%) and it was not due to proliferation of fungi and bacteria, because pure AA in aqueous solution showed a similar decrease while the solution kept clear.

Concluding, a freshly prepared fruit juice may be kept safe in the refrigerator at least for 7 days, with no apparent fall of the AA content or proliferation of microorganisms.

c) Action of H_2O_2 upon AA

Once convinced, (and contrary to what is believed), that pure AA in aqueous solution is relatively stable to atmospheric oxygen, we started to assay it after incubation with H_2O_2. For that, a fresh aqueous solution containing about 8.8 mg of AA/mL, pH = 3.0, was initially titrated and consumed 10.2 mL of 0.0515 N I_2/5.0 mL, corresponding to 9.2 mgAA/mL.

Fifty (50.0) mL of this solution was then incubated with 0.5 mL of a 29% (m/v) H_2O_2 during 24 h at 24°C. Titration consumed now 4.0 mL of the I_2 solution/5.0 mL corresponding to: 3.6 mgAA/mL = 39.4% of original value.

In parallel, the same incubation was carried out at pH = 10.0. After 24 h, the pH fell to 3.4 and 5.0 mL were then transferred again for titration after bringing the pH to 3.0. The V I_2 consumed now was only 0.5 mL (0.4 mg/mL = 4.9% of the original value), thus indicating almost complete oxidation of AA. In order to be sure that no H_2O_2 was still present and exclude the possibility of oxidation of I^- by H_2O_2:

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2\ H_2O,$$

the same titration was carried out after treating the incubated solution with 0.1 mL of fresh mouse blood at pH 7.5. Hemolysis of red blood cells releases catalase, which quickly promotes decomposition of H_2O_2 into $H_2O + 1/2\ O_2$. The V I_2 consumed now was 0.4 mL, corresponding to 0.3 mg/mL, the true residual content of ascorbic acid, 3.9% of the original value.

Concluding, stability of ascorbic acid against H_2O_2 decreases at high pHs. The high stability at pH 3 may be explained as a consequence of its chelated structure (BEYER; WALTER, 1996) as shown in Figure 2.

This chelation, which stabilizes the molecule, is expected to be destroyed at high pHs due to loss of H^+ ($pK_1 = 4.17$ and $pK_2 = 11.57$). In addition, the resulting negatively charged ascorbate

ion, disposing now a higher electronic density, becomes a more potent reducing agent:

$C_6H_8O_6 + OH^- + H_2O_2 \rightarrow C_6H_5O_6^- + 3\ H_2O$

Ascorbic acid Dehydroascorbate ion

In aqueous solution, the two carbonyl groups (in position 2 and 3) of dehydroascorbic acid assume the hydrated form $–C(OH)_2 – C(OH)_2$-, with $K_a = 12.6x10^{-5}$ and therefore $pK_a = 3.9$.

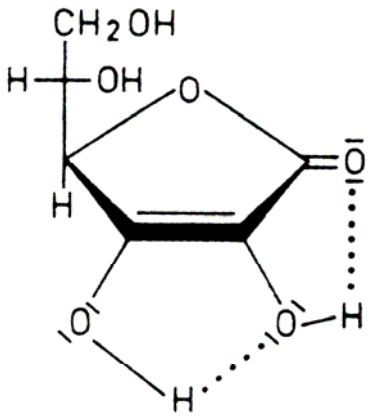

Figure 2. Chelated structure of L-ascorbic acid

A microtest was also performed, using Tillman's reagent (2,6–dichloroindophenol sodium salt hydrate) to confirm the presence of L-ascorbic acid, based on its ability to oxidize it to L-dehydroascorbic acid in acid solution. For that, a drop of an aqueous 0.1% (w/v) of the reagent was mixed with a drop of the test solution. In the presence of L-ascorbic acid, the blue reagent is changed into its colourless reduced form. When fruit juice is being assayed, attention should be paid regarding the original fruit colour.

Fe^{3+} as titrant of AA

As above reported, the quick fading of the I_3^--starch complex blue color in the evaluation of ascorbic acid in fruit juices is an inconvenient cause of error in the I_2 method. A new procedure, as described in Methods, was then developed to overcome this problem after excluding β-carotene, glucose and fructose as probable interferent components and concluding that high mol. weight (> 1,000) compounds were responsible for this slow side reaction.

A solution containing 13.55 g of $FeCl_3.6H_2O$ (mol. weigh = 270.3) + H_2O + 10 mL of concentrated HCl + H_2O q.s.p. 1000.0 mL (A), and a second one containing 0.441 g of pure AA + H_2O + 1 mL of concentrated HCl + H_2O q.s.p. 100.0 mL (B) were prepared. Solution B was titrated with 0.0515 N I_2, consuming 9.9 mL/10.0 mL. Therefore, N of AA = 0.0510 (mEq./mL)

Ten (10.0) mL of this solution + 20 mL H_2O + 0.2 mL of saturated SCN^- solution were used now to titrate solution A and consumed 10.2 mL of the Fe^{3+} solution (A). Therefore, N Fe^{3+} = 0.0500 (mEq./mL). This is the new titrant solution to be used and compared now with the I_2 solution in the evaluation of AA, as follows:

Fresh orange juice was sifted through a plastic sieve and then its ascorbic acid titrated with I_2 and Fe^{3+}.

Results were: 38.5 mg/100.0 mL by the I_2 method and 35.2 mg/100.0 mL by the Fe^{3+} method, therefore 91.4% of the first value. The same comparison for other fruits, including cashew (*Anacardium occidentale*), tangerine (*Citrus reticulata*) and strawberry (*Fragaria spp*), is shown in Table 4.

Table 4. Comparison between values, in mg/100.0 mL juice, of the AA concentration in several fruit juices, obtained by the I_2 method and the Fe^{3+} method.

Fruit	I_2 method mgAA/100.0 mL juice	Fe^{3+} method mgAA/100.0 mL juice
Orange	38.5 ± 0.42	35.2 ± 0.14 (91.4%)
Pineaple	18.1 ± 0.14	15.6 ± 0.00 (86.2%)

Cashew	285.7 ± 5.23	273.0 ± 0.70 (95.5%)
Tangerine	18.1 ± 0.00	17.6 ± 0.28 (97.2%)
Lemon	25.4 ± 0.28	23.8 ± 0.14 (93.7%)
Strawberry	33.6 ± 0.42	30.8 ± 0.00 (91.7%)

As shown, values for the iodine method were always higher than those obtained by the Fe^{3+} method. Even considering that the juice colour always composes the final tonality at the end point, the Fe^{3+} method looks more accurate since it avoids overestimation of AA due to side reactions.

CONCLUSIONS

Preliminary assays on the estimation of AA, by the I_2 method, in fruit juices, pointed to a persistent inconsistency of the blue color, at the end point, for all fruits analyused, which was attributed to the presence of high molecular weight (> 1,000) components of the fruit juice, since β-carotene (mol. weight 536.85), glucose and fructose (mol. weight 180.16) were excluded as hypothetic interferers. Ripe oranges revealed a higher contents (in mg/100.0 g) of AA than unripe oranges, what may be extended to other fuits.

Once observed that the classical iodine method for Vitamin C quantification affords overestimated values for all assayed fruit juices due to undesirable side reactions, a new method, replacing I_2 by Fe^{3+} as the oxidizing agent, is proposed to overcome this problem. Vitamin C is often referred to as unstable against atmospheric oxygen, thus leading to erroneous conclusions such as: fresh fruit juices should be consumed soon as to avoid oxidation; or bruised fruits should be discarded because their Vitamin C was already oxidized. Our results showed that, at 4°C in the dark, practically no Vitamin C is lost, along one week, in a pure L-ascorbic acid aqueous solution, as well as in a freshly-prepared acerola juice, both kept in loosely covered vessels. Proliferation of bacteria and fungi, which occurs at room temperature and under day light exposition in fruit juices, but not in the L-ascorbic aqueous solution, may be prevented by food preserving agents such as benzoic acid. An extensive (60.6%) oxidation of L-ascorbic acid, even at low pH, was shown to occur however at room temperature, under day light exposition, after 24 h in the presence of H_2O_2.

As seen, aside the myth, which perdures to this very day, that overdoses of Vitamin C is able to prevent the common cold, we had, but have not now, to face the myth that this vitamin is highly prone to oxidation by atmospheric oxygen.

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RESUMO: Este trabalho teve como objetivo uma avaliação do método clássico do iodo para a quantificação da vitamina C (ácido L-ascórbico) em sucos de frutas, assim como uma pesquisa da estabilidade desta vitamina tão popular sob diferentes condições de pH, temperatura e exposição à luz, além de uma proposta de novo método de quantificação.

Nossos resultados indicam uma persistente reversibilidade da cor azul do complexo amido-triiodeto no ponto final, quando usamos a clássica titulação com iodo, e uma super-estimação da verdadeira concentração da vitamina em sucos de frutas. Um novo método de dosagem é proposto a fim de superar este problema. Conclusões surpreendentes foram obtidas em relação à controvertida estabilidade do ácido L-ascórbico frente ao oxigênio atmosférico, em pH baixo, mesmo no suco da fruta e em temperatura ambiente, mostrando que o maior problema em relação ao envelhecimento de sucos de frutas é a proliferação de microorganismos e não a oxidação espontânea do ácido L-ascórbico.

PALAVRAS-CHAVE: Vitamina C. Oxidação-redução. Estabilidade.

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