

Oxidation time of Ascorbic Acid in two different types of Solutions

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Abstract— The article evaluated the survival function and time of failure (ascorbic acid oxidation) in natural fruit juices, comparing to oxidation of this vitamin in simple aqueous medium. The analysis showed that the oxidation in natural fruit juices (orange, barbados cherry and cashews) have failed quickly compared to simple aqueous medium.

Keywords— Survival Function. Risk ratio. Vitamin C.

I. INTRODUCTION

Antioxidants are chemical compounds that can prevent or reduce the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species, which include free radicals, that is, antioxidants have the ability to react with free radicals and thus restrict the harmful effects on the body. Antioxidant supplementation can be used in situations where normal body defense mechanisms are not sufficient to attenuate the harmful action of free radicals from metabolic activities (Picchio et al., 2013).

Natural substances exhibit antioxidant activities that help decrease the incidence of cardiovascular disease, inflammation, brain dysfunction, and delay early aging (ROCHA et al., 2013).

Fruits and vegetables contain many compounds with potential antioxidant activity, such as phytochemical antioxidants, including simple phenolic compounds, glycosides and flavonoids such as vitamins C and E. Vitamin C also helps the body maintain levels of vitamin E, liposoluble antioxidant, and also acts as an anti-stress agent, favoring the reduction of glucocorticoid rates (FERNANDES et al., 2013).

Most of the antioxidants present in citrus are vitamin C and polyphenols, especially flavonoids. Vitamin C provides protection against uncontrolled oxidation in the aqueous medium of the cell, due to its high reducing power. Polyphenols are substances with great power to neutralize the molecules of free radicals (KLIMCKAC; PATIL, 2007).

Vitamin C is the common name given to 2,3-enediol-L-gulonic acid which is a powerful antioxidant because it prevents oxidation, that is, the loss of electrons. The body has different antioxidant defense systems, however, when imbalance occurs in the antioxidant defense, there is an increase in the number of free radicals, a process known as oxidative stress. Ascorbic acid (vitamin C) molecules undergo oxidation before other molecules oxidize, preventing and protecting these other oxidation molecules (BIANCHI; ANTUNES, 1999).

It is known that during food storage and processing, most vitamins can be spoiled due to chemical reactions, especially by oxidation. This is a serious problem, especially in relation to the conditioning of natural foods, since the simple contact with the oxygen of the air during the storage time favors the oxidation reactions of the vitamins inherent in the composition of these foods (BRITO et al.) Foods like cashew, acerola and orange have high amounts of ascorbic acid, and are generally ingested by man in the form of juices. As soon as the juice of these fruits is extracted, the process of oxidation of vitamin C by the oxygen of the air begins, which means that in the course of time the amount of ascorbic acid present in the juice decreases. Oxidation may be represented by the conversion of ascorbic acid to dehydroascorbic acid as shown in

Figure 1.

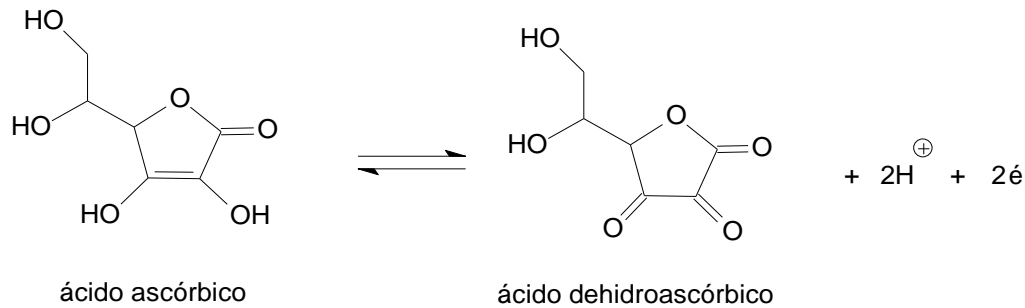


Fig.1: Oxidation of ascorbic acid to dehydroascorbic acid.

Source: Organized by the authors.

In this sense, the study of the failure time, or the occurrence of the oxidative process of ascorbic acid, in different solutions and in ambient conditions, allows to evaluate packaging and preparation techniques, in order to optimize its use and consumption. Therefore, the present study aims to evaluate the failure time and risk function of ascorbic acid oxidation in natural fruit juices, comparing to the oxidation of this vitamin in a simple aqueous médium

II. MATERIALS AND METHODS

2.1. Sample Preparation Planning and Procedures

To carry out the analyzes, oxredution titrations were made using iodine solution (I₂) 0,01 molL⁻¹ as titrant and starch (1%) as indicator of the turning point. The iodine (I₂) acts as an oxidizing agent, causing oxidation of the ascorbic acid contained in the solution to dehydroascorbic acid. Initially the solution is colorless, but when there is excess iodine, ie all ascorbic acid has been oxidized, this solution turns blue, indicating the end of the titration.

For determination of the ascorbic acid content in orange juice, 25.0 ml of orange juice was pipetted and transferred to a 100.0 ml volumetric flask and the volume was quenched with distilled water. Then 25.0 mL of this solution was pipetted into a 125.0 mL Erlenmeyer flask and 5.0 mL of 1% starch indicator solution (m / m) was added. The solution was titrated with 0.01 molL⁻¹ iodine until a permanent blue color appeared. The concentration of ascorbic acid present in the juice over 2.5 h was calculated and then compared with the other results obtained.

Regarding the determination of the oxidation rate of ascorbic acid present in aqueous médium, the same initial amount of ascorbic acid PA present in orange juice solubilized in distilled water was weighed and transferred to a volumetric flask of 1000.0 ml and the volume completed with distilled and deionized water. 25.0 mL of

this solution was then pipetted to a 125.0 mL Erlenmeyer flask, thereby initiating the titration. The concentrations of ascorbic acid present in the aqueous médium over 2.5 h were determined at each 30 minute interval and under magnetic stirring at 7,000 rpm. Then, they were compared with the results obtained in the oxidation of ascorbic acid present in natural fruit juice.

In the analysis of ascorbic acid present in acerola juice, 252.0 g of fruit were used, which produced 125.0 mL of juice after extraction. The juice diluted with 200.0 mL of water, raising to a total volume of 325.0 mL of solution. From this solution were pipetted 25.0 mL and transferred to a volumetric flask of 100.0 mL, completing with distilled water. Then 25.0 mL were pipetted from the volumetric flask to a 125.0 mL Erlenmeyer flask and titrated.

Regarding the determination of the oxidation rate of ascorbic acid present in aqueous médium, the same initial amount of ascorbic acid present in acerola juice was weighed, dissolved in water and transferred to a 1000.0 mL volumetric flask and filled with deionized water. Then 25.0 mL was pipetted into a 125.0 mL Erlenmeyer flask and titrated with 0.01 mol L⁻¹. The concentrations of ascorbic acid present in the aqueous médium in the course of 2.5 h were determined by the same procedure, and then compared with the results obtained in the oxidation of the natural juice.

For the analysis of cashew juice, 440.0 g of the fruit were used, which provided a volume of pure juice, after filtration maceration, of 437.6 mL. To this juice was added 150.0 mL of water, thus raising to a final volume of 587.6 mL of solution. From this solution were pipetted 25.0 mL and transferred to a volumetric flask of 100.0 mL, completing with distilled water. Then 25.0 mL were pipetted from the volumetric flask to a 125.0 mL Erlenmeyer flask and titrated.

Ascorbic acid content of the cashew juice and in aqueous médium were weighed, the same initial amount of ascorbic acid present in the cashew juice was weighed,

dissolved in deionized water and transferred to a 1000.0 mL volumetric flask and volume with distilled water. Subsequently, 25.0 mL were pipetted and transferred to a 125.0 mL Erlenmeyer flask and titrated with 0.01 mol L⁻¹ I₂ solution. The concentrations of ascorbic acid present in aqueous medium in the course of 2.5 h were determined and calculated by the same procedure, and then compared with the results obtained in the natural juice analysis.

The determination of ascorbic acid concentration as a function of the time of exposure to air was carried out at intervals of 30 minutes in triplicates for a period of 2.5 hours, that is, 150 minutes exposed to oxygen from the

air, under agitation at 7,000 rpm. To calculate the mass of ascorbic acid present in each solution, the following procedure was used:

$$\text{Mass of Vitamin C} = M * V * MM, \text{Equation 1}$$

where, M is the molarity of iodine (0.01 molL⁻¹); V is the volume spent in liters of iodine in titration and MM is the molar mass of ascorbic acid (176 mols / g).

In Table 1 the spent volumes of iodine in each titration are described. It should be noted that the volumes of 0.01 molL⁻¹ spent I₂ solution are obtained by the average of three titrations (triplicate) performed.

Table 1: Volumes used of I₂ (0,01 molL⁻¹) in the titrations of preparation of the samples with ascorbic acid, in simple aqueous solutions (SAS) and in natural fruit juices (SNF).

Time (min)	Volume spent (in ml) on the titrations of the simple aqueous solutions			Volume spent (in mL) spent on titrations of natural fruit juices		
	Orange	Acerola	Cashew	Orange	Acerola	Cashew
0	2,65	39,50	7,25	2,65	39,50	7,25
30	2,38	34,80	5,60	2,50	35,55	6,50
60	1,98	31,08	5,24	2,20	31,70	5,85
90	1,78	26,80	4,50	2,00	28,50	5,30
120	1,56	24,38	3,91	1,80	27,00	4,90
150	1,45	23,21	3,26	1,65	26,50	4,35

Source: Prepared by the authors.

2.2. Statistical treatment of collected data

After the data collection, a statistical study was performed with the measurements of ascorbic acid concentration (g / L) in each of the experimental samples. The aim is to demonstrate the failure performance (decrease of the ascorbic acid concentration function) over the time measured in minutes. The log-normal distribution of sample reliability was obtained from the probability density function (pdf), given by:

$$F(t) = \frac{1}{\sigma \sqrt{2\pi}} \exp\left(-\frac{(\ln(x)-\mu)^2}{2\sigma^2}\right), \text{Equation 2}$$

where, μ are the parameters obtained in each sample and, σ is the scale of the parameters evaluated. The estimates of the likelihood function of the parameters are estimated by the maximum likelihood procedure (GIESBRECHT; KEMPTHORNE, 1966).

As for the survival function S (t), which represents in the experiment the probability of an "ascorbic acid" unit of concentration "surviving", above the time interval in t (minutes), is obtained naturally by:

$$S(t) = 1 - F(t), \text{Equation 3}$$

and the confidence limits for a given estimate of the function S (t), is obtained by:

$$S_L(\hat{Z}) = S(\hat{Z}_2), \text{to the lower limits; Equation 4}$$

$$S_U(\hat{Z}) = S(\hat{Z}_1), \text{to the upper limits Equation 5}$$

Where,,

$$Z_1 = \hat{z} - z_\alpha \sqrt{\text{Var}(\hat{z})}; \text{Equation 6}$$

$$Z_2 = \hat{z} + z_\alpha \sqrt{\text{Var}(\hat{z})}, \text{Equation 7}$$

in which, $z_\alpha = (1 + \alpha) / 2$ for the critical value of confidence, given in a standardized normal distribution (RIGDON; BASU, 2000).

Regarding the variance of the survival probabilities (survival probabilities) of the samples, it is given by:

$$\hat{z} = \frac{x - \hat{\mu}}{\hat{\sigma}}, \text{Equation 8}$$

or yet,

$$\hat{z} = \frac{\ln x - \hat{\mu}}{\hat{\sigma}}, \text{Equation 9}$$

And, therefore, for the log-normal distribution, one has:

$$\text{Var}(\hat{z}) = \frac{\text{Var}(\hat{\mu}) + \hat{z}^2 \text{Var}(\hat{\sigma}) + 2\hat{z} \text{Cov}(\hat{\mu}, \hat{\sigma})}{\hat{\sigma}^2} \text{Equation 10}$$

In sequence, the instantaneous failure rate (or hazard rate) was obtained for each time t (in minutes), as shown in the hazard function. It is possible to consider in this specific study, in which phase of experimental life, the probability of occurrence of failure is greater or lesser for each sample group, and also the expectation of its duration (or instantaneous failure rate at a given moment, t)

The risk function is given by:

$$h(t) = \frac{f(t)}{1-F(t)}, \quad \text{Equation 11}$$

where f (t) and F (T) are cdf (cumulative distribution function) and pdf (probability density function), respectively, of the chosen distribution (MEEKER and ESCOBAR, 1998). The Reliability / Survival Analysis package and the Parametric Distribution Analysis-Right

Censoring function of the MINITAB software (version 17) were used for the statistical study.

III. RESULTS AND DISCUSSION

After the determination of the ascorbic acid content in fruit juices, a comparison was made of the oxidation rate of ascorbic acid by air oxygen in fruit juices and also simultaneously a simple aqueous solution containing initially at the initial time, the same amount of ascorbic acid in fruit juices.

From the data presented in Table 2, it can be seen that the oxidation rate of ascorbic acid was higher when compared to the same concentration of ascorbic acid in fruit juice. It is noted that, for example, for orange juice, a concentration of 0.746 g / L of ascorbic acid was exposed prior to oxidation

Tabela 2: Concentrations of ascorbic acid present in simple aqueous solutions (SAS) and natural fruit juices (SNF), at different times evaluated in the study.

Time (min)	Concentration of ascorbic acid (g / L) in single aqueous solution			Concentration of ascorbic acid (g / L) in natural fruit juices		
	Orange	Acerola	Cashew	Orange	Acerola	Cashew
0	0,746	28,920	2,739	0,746	28,920	2,739
30	0,670	25,479	2,117	0,704	26,028	2,458
60	0,557	22,756	1,981	0,619	23,209	2,212
90	0,501	19,622	1,702	0,563	20,866	2,004
120	0,439	17,850	1,478	0,506	19,768	1,853
150	0,408	16,994	1,233	0,465	19,402	1,645

Source: Prepared by the authors.

When subjected to oxidation, the concentration of ascorbic acid found after 30 minutes of stirring was 0.704 g / L, whereas in the simple aqueous solution of ascorbic acid, from the initial concentration of the same 0.746 g / L, a decrease was observed to 0.670 g / L after 30 minutes. This higher oxidation in the simple aqueous solution of ascorbic acid, compared to the oxidation of the ascorbic acid present in the fruit, was observed at all determined times, according to Table 2.

As for the statistical study of the failure time, for samples of Orange in simple aqueous solution (SAS) and in natural fruit juices (SNF), it was noticed that those in aqueous solution failed before and unlike the samples in natural fruit juices (Figure 1). The hazard function indicates that the probability of occurrence of failure (oxidation of ascorbic acid) in the samples in natural juices occurs late, already in the final phase of experimental evaluation.

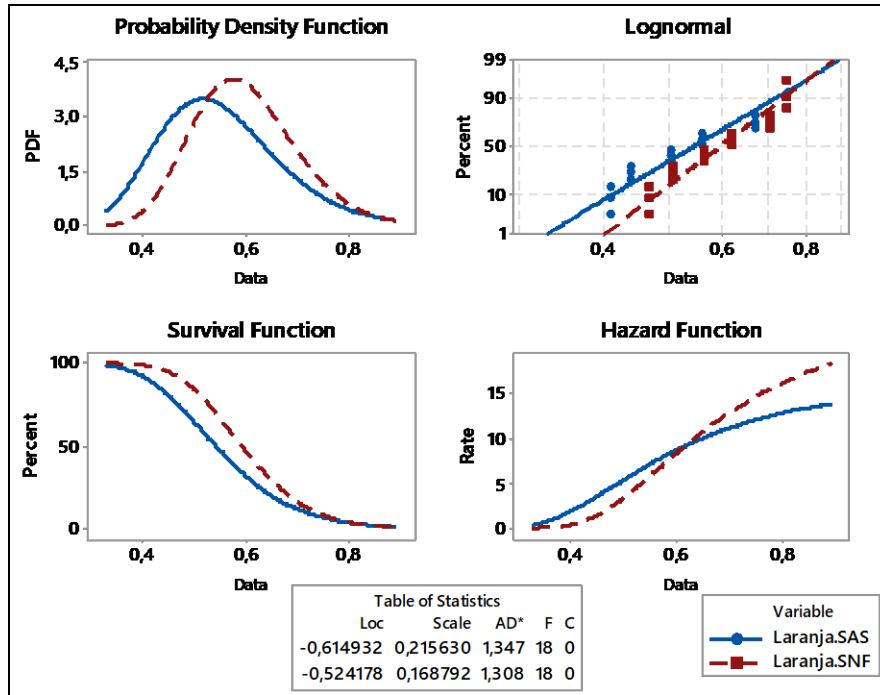


Fig.1: Distribution of the estimates by the maximum likelihood criterion of the complete ascorbic acid (g / L) oxidation data for triplicate samples of orange in single aqueous solution (SAS) and in natural fruit juices (SNF).

Source: Prepared by the authors.

The same considerations can be attributed to the other experimental samples, containing acerola and cashew in simple aqueous solution and in natural fruit juices. In the acerola samples, the survival distribution and the risk rate function are quite similar with the samples containing orange (Figure 2).

In general, the characteristic of the solution is determinant for the distribution of the ascorbic acid concentrations, throughout the time of evaluation of the experiment. The probability of oxidation of ascorbic acid, which is already close to the final phase of the experiment, is more easily perceived from the risk ratio function for the two samples (orange and acerola).

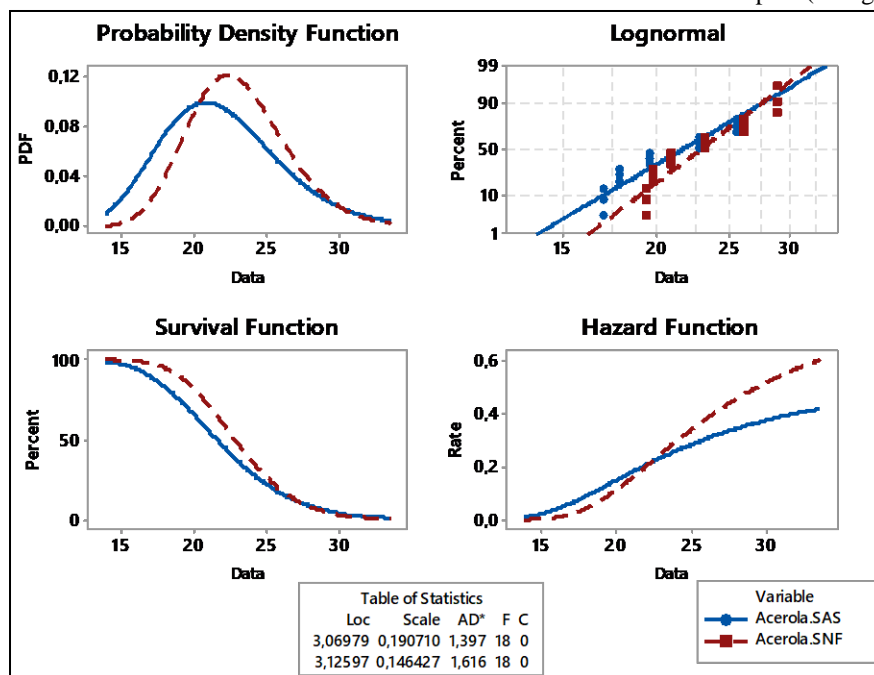


Fig.2: Distribution of the estimates by the maximum likelihood criterion of the complete ascorbic acid (g / L) oxidation data for the triplicate samples of acerola in single aqueous solution (SAS) and in natural fruit juices (SNF).

Source: Prepared by the authors.

Likewise, for Cashew samples in simple aqueous solution and in natural fruit juices, it was observed that those in aqueous solution failed before and unlike samples in natural fruit juices (Figure 3). The hazard function (Hazard Function) has demonstrated that the probability of occurrence of failure in the samples in natural juices is also later in the final phase of experimental evaluation, contrary to what happens in the initial phase of the experiment.

It should be noted that the greatest difference between the distribution of data evaluated in the samples, considering the probability of survival, more extensive in

the treatments with natural fruit juices in relation to the aqueous medium, and in relation to the risk rate function.

For this set of samples (using cashew), the probability of occurrence of failure (oxidation of ascorbic acid) in simple aqueous solution is much less pronounced and, at least visually, a more discrete distribution. Compared to the other samples (orange and acerola), the distance between the hazard rate function in the final phase of the experiment is much wider for the two solution models (aqueous solution versus solution with fruit juice).

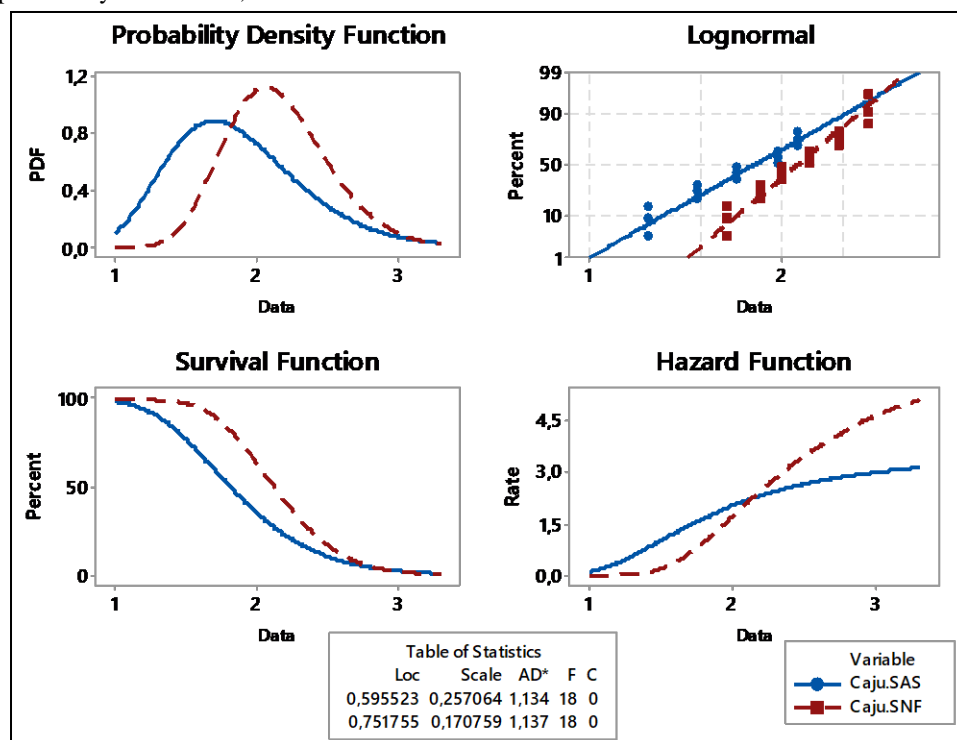


Fig.3: Distribution of the estimates by the maximum likelihood criterion of the complete ascorbic acid (g / L) oxidation data for triplicate samples of cashew nuts (SAS) and natural fruit juices (SNF).

Source: Prepared by the authors.

Therefore, the simple aqueous solution allows for greater oxidation of ascorbic acid in the different types of fruits used in the experiment compared to the solution containing natural fruit juice. The oxidation rate, however, is more accentuated at the end of the experiment, using orange and acerola, compared to cashew nuts.

IV. CONCLUSIONS

The present study allowed to conclude that:

- all the juices used in the experiment (orange, acerola and cashew), in simple aqueous solutions, failed very quickly when compared to samples in solution of natural fruit juices.

- There is a presence of natural antioxidants in fruits that prevent the oxidation of ascorbic acid in dehydroascorbic acid as fast as its oxidation in its pure form, ie without the action of these natural antioxidants.
- acerola juice had a higher ascorbic acid content, and demonstrated a higher failure rate (ascorbic acid oxidation) during the 150 minutes of the experiment, indicating that there is a greater antioxidant "protection" of vitamin C in orange and cashew juice in relation to acerola juice.
- the characterization of these natural antioxidants in orange, cashew and acerola is proposed in future studies, considering the ability to retard the rate of

oxidation of ascorbic acid in order to attenuate the harmful effects of oxidative stress intrinsic to our body's metabolic activities.

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