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Kinetic study of ascorbic acid degradation from grapes

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Abstract The aim of this study was to investigate the effect of storage at -4^{0}C on the ascorbic acid (vitamin C) content of grapes (*Vitis vinifera*) for 1, 2, 5 and 8 months. A sensitive UV-VIS spectrometric method based on Prussian Blue reaction was applied for vitamin C determination and some kinetic calculations were presented. The varieties of red and white grapes used were from Murfatlar vineyard: Mamaia, Cristina and Columna. Fresh samples before storage and samples after one, two, five and nine month of storage in the freezer were investigated. The half time values of ascorbic acid degradation are between 160.78 and 232.23 hours, respectively between 2.9×10^{-3} hours^{-1} and 4.3×10^{-3} hours^{-1} for constant rate. It was observed that the concentration of ascorbic acid from studied grapes decreases with the increase of the storage time.

Keywords: kinetic study, ascorbic acid, grapes, Murfatlar vineyard, Prussian Blue reaction

1. Introduction

Consumption of fruit and vegetable juices containing bioactive compounds such as ascorbic acid (AA) can reduce various degenerative diseases [1, 2].

Vitamin C is instable thermic and therefore in fruit and vegetables it provides an indication of the loss of other vitamins and acts as a valid criterion for other sensorial or nutritional components, such as natural pigments and aromatic substances. Its concentration decreases during storage, depending on storage conditions, such as temperature, oxygen content and lights [2].

Ascorbic acid has an essential role in many biological processes. [3] Its strong reducing properties have often been exploited to establish a variety of methods for its kinetic-based determination. As results, some recent spectrophotometric procedures rely only on monitoring the advance of a simple redox reaction, e. g. the reduction of toluidine blue [4, 5], ammonium molybdate [6], EDTA-Co(III) [7], or 2,6-dichlorophenolodophenol [8]. Other techniques use either the catalytic [9, 10] or inhibiting [11-13] effect of ascorbic acid on the enhancing of different systems. Some combine the advantages of both flow-injection analysis (FIA) and kinetic spectrophotometric determination to achieve sampling rates up to 100 h^{-1} [13]. Reported enzymatic [14] or porphyrin - formation [15] indicator reactions also use FIA.

It was also studied the half life and the changes in AA of amaranth and fenugreek during low cost storage using simple packaging method (titration with 2,6 dichlorophenol indophenols dye) [16].

Other researchers were evaluated the effect of pulsed electric fields (PEF) and conventional pasteurization (90^{0}C, 20s) on AA content of orange juice, and they was assessed modifications in AA concentration of orange juice stored in refrigeration at 2 and 10^{0}C for 7 weeks [2].

Another kinetic study presents the dependence of the stability of AA on the pH of the mixture of acetonitrile with a phosphate buffer solution and on the concentration of acetonitrile in this mixture [17].

The objective of the present work was to determine the ascorbic acid concentration in some red and green grapes (*Vitis vinifera*) from own sort of Murfatlar vineyard. It was determined the AA degradation after one, two, five and eight months of storage at cold (-4^{0}C). It was used a spectrophotometric method which was optimized in a previous paper [18, 19].

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Were studied samples from three varieties of Murfatlar grapes: Cristina, Mamaia and Columna.

2. Experimental

Reagents and solutions

All reagents were of analytical-reagent grade (Merck and Fluka) and all solutions were prepared using distilled-deionized water.

For the spectrophotometric method the following reagents were prepared: Fe (III) reagent ($FeCl_3$) in the $2.0x10^{-3}$M concentration (by Merck), the hexacyanoferrate (III) solution $2.0x10^{-3}$M (by Fluka), KCl 0.1M and respectively 0.01M HCl (by Merck).

Instrumentation

The weightings were made at a Metler Toledo analytical balance with ±0.0001g accuracy.

For absorbance measurements an UV-VIS Thermospectronic HR 200 spectrometer from Heλios with 10mm cells was used.

Sample preparation

Each variety of grapes (Mamaia – red grapes, Cristina – red grapes and Columna- white grapes) from a diagonal parcel of Murfatlar land was collected just before ingathering. This vintage was made at noon to avoid the temperature differences between day and night. A number of 100 grain grapes were selected after the collection of 300 grain grapes from the cluster, to homogenize the sample for each variety of grapes. The samples were squeezed, then the juice was filtered and the filtrate was used for the determination of ascorbic acid. In the case of red grape samples it was necessary to use the animal black coal for the discoloration of filtrate before determinations.

Spectrophotometric procedure

The reagents: $2.0x10^{-3}$M Fe (III) chloride salt (1mL), $2.0.x10^{-3}$M potassium hexacyanoferrate (III) solution (1mL), 0.1M KCl (1mL), 0.01M HCl (1mL) and the samples (1mL) were mixed and fill up to 50mL in calibrated flasks with distilled water. After 10 minutes absorbance of the complex was read at the 700nm. The calibration curve was linear over the range 0.0880-0.7040mg/L ($5x10^{-5}$-$4x10^{-4}$M) with a correlation coefficient of 0.9994 [19].

For each sample three determinations were performed and average results are reported.

Kinetic procedure

For the kinetic study the rate constant and the half-life for Vitamin C there were determinated. Considering that the Vitamin C degradation is a first order reaction for rate constant determination was used the Eq. (1), and for half-life Eq. (2):

$$k = \frac{2,303}{t} log \frac{a}{a-x} \qquad (1)$$

$$t_{1/2} = \frac{0.693}{k} \qquad (2)$$

where:

k = the rate constant ($hours^{-1}$);
t = the interval of time since the reaction has began (hours);
a = the initial concentration (mgAA/100g grapes);
a-x = the concentration at different time of reaction (mgAA/100g grapes);
$t_{1/2}$= the reaction's half-life (hours);

3. Results and discussions

The aim of this study was to investigate the effect of storage at -4^0C on the AA content of grapes for 1, 2, 5 and 8 months.

The values of vitamin C concentrations were between 2.03 and 0.48 mgAA/100g grapes for Columna varieties, between 1.72 and 0.32 mgAA/100g grapes for Cristina and for Mamaia between 1.48 and 0.76 mgAA/100g grapes.

It was observed that the vitamin C concentration was decreasing further that at half after eight months of storage in the freezer for two varieties of grapes: Columna and Cristina. For Mamaia varieties of grapes the vitamin degradation was lower (Fig. 1).

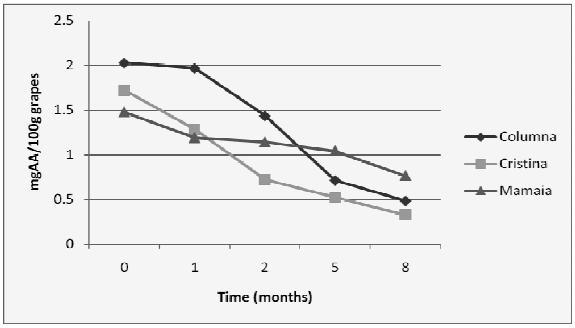

Fig.1 Variation of vitamin C concentration with time for Murfatlar varieties of grapes.

The results obtained by other Romanian researchers in fresh grape juice (50mgAA/L) [20, 21] were higher than the results reported in this paper from own varieties Murfatlar grapes (1.48 and 2.03 mgAA/100g grapes).

There were however differences between the three different varieties of grapes. AA content of grapes is positively influenced by light intensity during growth [22] and negatively influenced by climacteric conditions (rain) [18].

For kinetic calculations, time 0 was considered the time before storage at low temperature.

It is generally assumed that the degradation of vitamin C follows first-order kinetics. The stability of vitamin C in food products is totally determined by their residual oxygen level, and its degradation occurs in two different conditions, aerobic and anaerobic. In aerobic conditions, ascorbic acid oxidizes to dehydroascorbic acid followed by hydrolysis and oxidation to form diketogulonic acid and oxalic acid. In anaerobic conditions there is a series of dehydrations and hydrolyses, finally giving furfural and carbon dioxide. Lin and Agalloco [23] comment that the rate equation of ascorbic acid degradation is first order when oxygen is present in abundance (for aerobic degradation) or if it is totally excluded (for anaerobic degradation).

Storage of grapes at low temperature did not significantly decrease the ascorbic acid content over the first storage period but the higher decrease was observed with the increase of time.

The estimated regression equations between log ascorbic acid contents and storage time were showed in Fig.2-4.

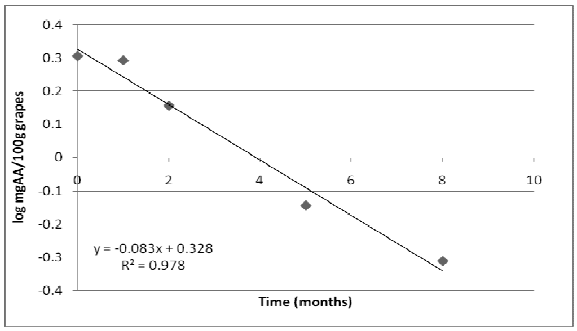

Fig.2 Effect of storage at low temperature on log ascorbic acid content of Columna grapes

The rate of degradation of vitamin C depends on the temperature which is a critical factor involved in its destruction. So, it can be observed that the longer the storage time at low temperature, the higher the increase in ascorbic acid loss.

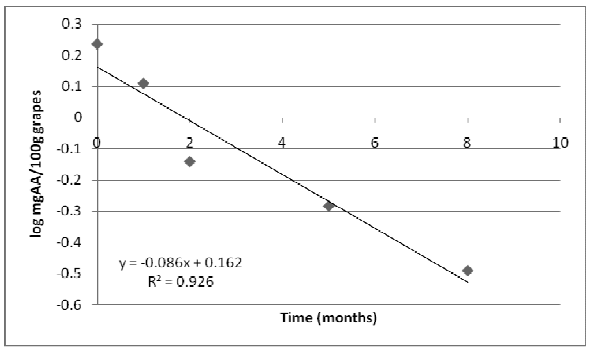

Fig.3 Effect of storage at low temperature on log ascorbic acid content of Cristina grapes

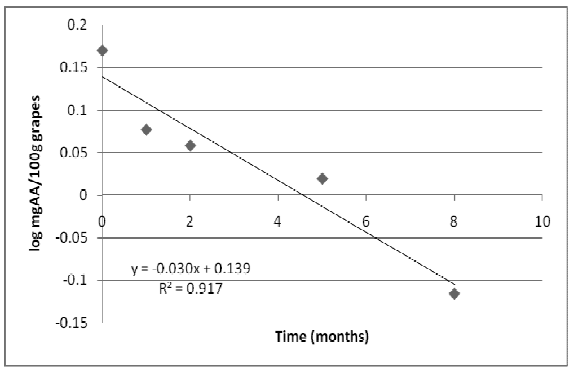

Fig.4 Effect of storage at low temperature on log ascorbic acid content of Mamaia grapes

Table 1. Rate constants and half time of ascorbic acid degradation from different types of grapes

Time (hours)	Rate constant (hours^{-1})			$t_{1/2}$ (hours)		
	Columna grapes	Cristina grapes	Mamaia grapes	Columna grapes	Cristina grapes	Mamaia grapes
744	$3.2\cdot10^{-3}$	$3.9\cdot10^{-3}$	$4.2\cdot10^{-3}$	210.57	173.42	162.67
1464	$2.9\cdot10^{-3}$	$3.5\cdot10^{-3}$	$3.4\cdot10^{-3}$	232.23	194.66	199.71
3672	$3.4\cdot10^{-3}$	$3.2\cdot10^{-3}$	$4.3\cdot10^{-3}$	200.23	216.63	160.78
5784	$3.4\cdot10^{-3}$	$3.7\cdot10^{-3}$	$3.9\cdot10^{-3}$	203.16	186.14	175.44

Storage at low temperature reduces the rate of oxidation of ascorbic acid to dehydroascorbic acid.

The values of rate constants and half times of ascorbic acid degradation from different types of grapes were presented in table 1.

One can observe that the rate constant for acid ascorbic content in grapes is almost unchanged during all the period. That means that the reaction order and the velocity are remaining constant during the 9 months and the decomposition process is taking place with a constant speed.

The half-time values show us a good stability of ascorbic acid even after refrigerated storage.

A study related to the determination of the rates of vitamin C loss in clear orange juice concentrate during storage for 19 weeks at temperatures of 4-24^{0}C, showed that the rate of ascorbic acid degradation at 24^{0}C was approximately 65.2 fold higher than the rate at 4^{0}C.

A loss of 70.2% ascorbic acid was noted during the 3 month storage period. The decrease in the ascorbic acid content might be due to various factors which affect the stability of ascorbic acid. These factors include temperature, presence of oxygen in the headspace and light [24].

Klimczak et al. [25] studied the effect of time and temperature of storage on vitamin C content of commercial orange juices obtaining that after 6 months of storage at 18, 28 and 38 °C, the content of vitamin C decreased by 21, 31 and 81%, respectively. Esteve et al. [26] studied the stability of ascorbic acid in fresh orange juice and commercial orange juices maintained at 4 and 10 °C, finding that at 4 °C the loss of ascorbic acid was <10% after 7 days of storage. Choi et al. [27] found that, for pasteurized juice (90 °C, 90 s), during refrigerated storage (4.5 °C) more than 50% of the ascorbic acid was lost within 3 weeks of storage, and it was completely degraded after 5 weeks of storage.

4. Conclusions

The ascorbic acid determinations were made to study the variations of concentrations of this vitamin in one, two, five and eight months after ingathering and after the grapes were kept at -4^{0}C.

The half-time values show a good stability of ascorbic acid even after refrigerated storage. Storage of grapes at low temperature did not significantly decrease the ascorbic acid content over the first storage period but the higher decrease was observed with the increase of time.

One can observe that the rate constant for acid ascorbic content in grapes is almost unchanged during all the period. That means that the reaction order and the velocity are remaining constant during the 9 months and the decomposition process is taking place with a constant speed.

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