

SPECTROPHOTOMETRIC EVALUATION FOR THE STABILITY OF THE ASCORBIC ACID FROM THE SWEET BRIAR EXTRACT (*ROSA CANINA*) AND WHITE SEA BUCKTHORN (*HYPPOPHAE RHAMNOIDES*)

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The nutritional value of the products obtained from sweet briar and white sea buckthorn comes from the complexity of their chemical composition. It is remarkable that these fruits contain such a great concentration of ascorbic acid (AA). Our researches on this matter show that the ascorbic content is higher in proportion with the white sea buckthorn than in the sweet briar (sweet briar: 190 mg/100g versus white sea buckthorn: 618mg/100g dry matter). At the same time our research points out the usefulness of the modern extraction methods used to obtain the extracts of sweet briar and white sea buckthorn. The purpose of the paper is also to study spectro-photometrically, on a pure system and from the mentioned extracts, the ascorbic acid stability. Consequently, we studied the dynamics of the ascorbic acid towards different solvents used in extraction: water, trichloroacetic acid (TCA) and acetic acid both in the presence and in the absence of light. On the other hand, the influence of the pH variation on the ascorbic acid stability was taken into account, both towards the pure solution of ascorbic acid and towards the extracts of sweet briar and white sea buckthorn. The spectrophotometric experimental results pointed out the high stability of the ascorbic acid when the extraction reactive was the acetic acid. In this context, the stability of the ascorbic acid from the extracts of sweet briar and white sea buckthorn, towards the acetic acid used as extraction reactive proved to be the best at pH = 2,903 for white sea buckthorn and at pH = 3,264 for sweet briar, in the absence of light.

Keywords: ascorbic acid, sweet briar and white sea buckthorn extract, spectrophotometric study

1. Introduction

The ascorbic acid is an essential nutrient, involved in the production of certain substances that allow the transmission of nervous influx, and the functions that facilitate the Fe absorption at the digestive tract level (Acatrinei, 1991). A fundamental chemical feature of the ascorbic acid (AA) is its redox behavior. Creutz (1981) showed that the reactions of oxidation and reduction of L-ascorbic acid is much more complicated due to the simultaneous reactions of proton transfer taking place.

As a participant in hydroxylation, the ascorbic acid is necessary for the production of collagen in the connective tissue. These fibers are present throughout the body, giving providing a stable but flexible structure (Burzo, 1994).

Ascorbic acid is found naturally in a wide range of plants. The human body does not produce vitamin C, therefore the only source of vitamin C for humans is the food based on fruit and vegetables (Parvu, 2006). In the present work, ascorbic acid stability in different conditions is studied. Also, dosages of ascorbic acid from sweet briar and white sea buckthorn was established

2. Materials and methods

2.1. Reagents and apparatus

In determining ascorbic acid dosage use, was made of analytical purity reagents: acetic acid solution of 10% buffer solution with pH 6.9 - 7 from 120 ml of NaH₂PO₄ (9.078 g/l) and 180 ml Na₂HPO₄ • 2H₂O

solution (11.786 g/l) standard solution of ascorbic acid (AA) M/1000; Tillmans reagent - 2,6-dichlorophenolindophenol M/1000 (Florea, 2008).

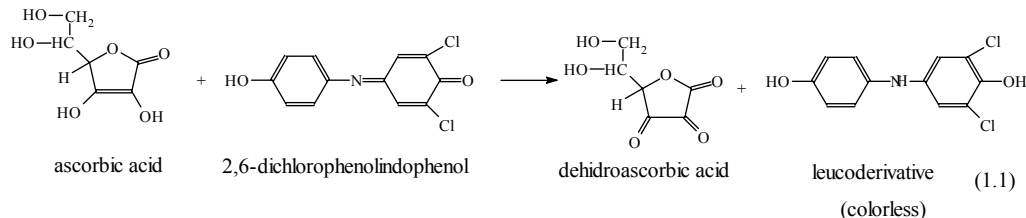
Measurement of pH was done with the multiparameter CONSORT C - 862. Spectra were recorded and processed with the spectrophotometer Spectro UV-VIS Double Beam PC 8 Auto Scanning cell UVD-3200, Lobomed, INC. For volumetric determination a microburette was used.

2.2. Processing the vegetal material

For the dosage of the ascorbic acid it was used dried plant/vegetal material: sweet briar and sea buckthorn. In a mortar the plant tissue is weighed with 10 mL of 10% acetic acid. It is grinded with quartz sand, filtered through wool and the clear solution is cooled into a scaled balloon 100 mL of bidistilled water.

2.3. Determination of ascorbic acid

The method of quantitative determination is based on selective oxidation of ascorbic acid to dehydroascorbic acid with 2,6-dichlorophenolindophenol (Enescu and Oita, 1990) (1.1).



3. Results and discussion

3.1. Dynamics of ascorbic acid from different solvents

To extract AA from the plant material several solvents have been tried: acetic acid, trichloroacetic acid (TCA) and water. It was also verified the stability of AA in time.

By spectrophotometering the solutions of ascorbic acid in the solvents mentioned above, of well-determined concentration, at specified time intervals, the spectra as in Figure 1 were obtained.

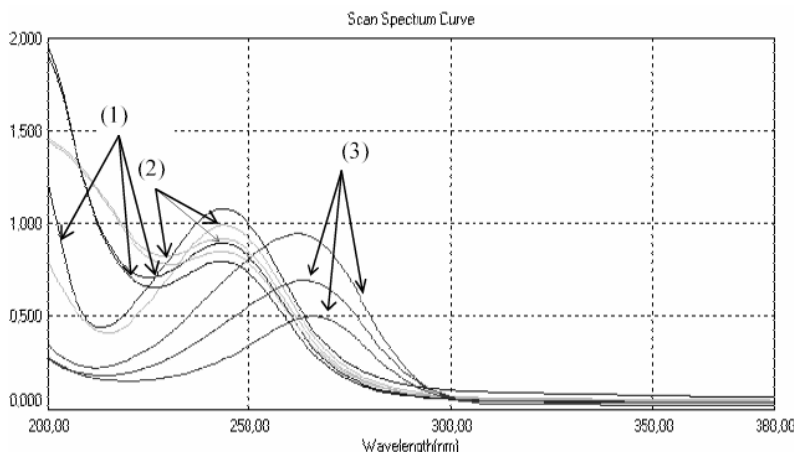


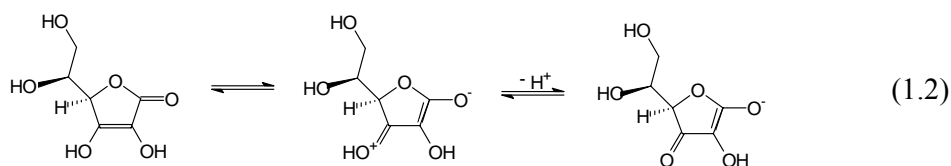
Figure 1. UV absorption spectra of ascorbic acid in solution of TCA (1), in acetic acid solution (2) and in water (3) at initial moment, after 30 min and after 60 min

From Figure 1 it can be seen that the greatest stability of ascorbic acid occurs in acetic acid; 60 minutes after the first recording of the absorption spectrum it is found that absorbing in ascorbic acid solution of acetic acid (0.993 initially) decreases little (0.757 in the end) as compared with that of the solution of

ascorbic acid in water (from 0.948 to 0.5) and TCA (from 1.084 to 0.701). This result was decisive in choosing the conditions for AA extraction from the plant material AA subject to analysis (Sorin, 1979).

3.2. Influence of pH variation on the stability of AA

The ascorbic acid has one of the most unusual structures with a nucleus of five atoms of lactone type that determines the bi-functionality of the compound both as alchene and as diol, a structural feature that, depending on the pH, makes the compound to stabilize one of its resonant structures and therefore to be either reducing or oxidizing or even having radical properties. The ascorbic acid in aqueous solution is in equilibrium with the dehydrogenated structure corresponding to the dehydroascorbic acid.



Depending on the concentration of H_3O^+ ions in the system, ascorbic acid will exist in two forms: non dissociated and dissociated. The concentration of dissociated form increases with increasing pH. When the pH is acid in the system, the non dissociated form will be dominant, H_2Asc the ascorbic acid, and, at alkaline pH the dehydroascorbic acid will prevail, $HAsc^-$ (Creutz, 1981).

To highlight the ascorbic acid at different pH there were prepared solutions of ascorbic acid of a concentration of 10^{-4} mol/l, which were adjusted to pH 4.7, 7.0 and 9.0 respectively with buffer solutions. The samples were obtained by spectrophotometry in the range 200-400 nm. The spectra from Figure 2 show the different behavior of ascorbic acid depending on pH of the reaction environment.

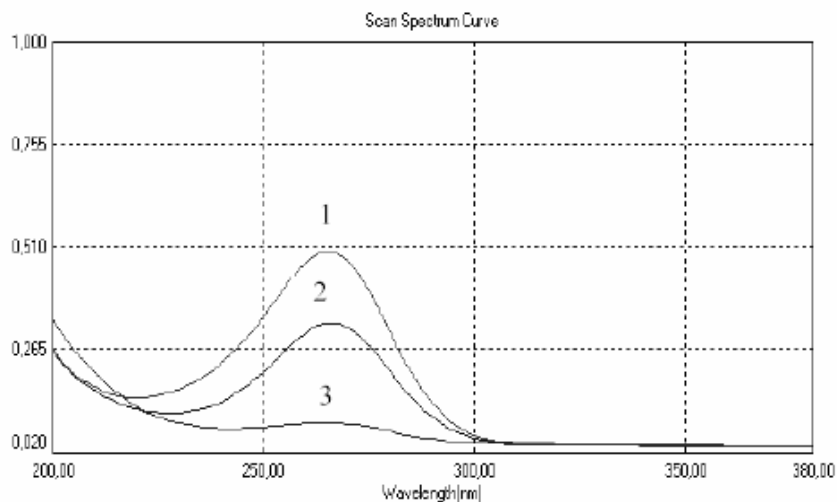


Figure 2. UV absorption spectra of ascorbic acid in aqueous solution 10^{-4} M at pH = 4.7 (1) at pH = 7 (2) at pH = 9 (3)

3.3. Dynamics of ascorbic acid in sweet briar and dry sea buckthorn

The extracts obtained by processing sweet briar and sea buckthorn fruits were analyzed by spectrophotometry UV-VIS (the results don't show the VIS absorption spectra). To highlight the

ascorbic acid suitable dilutions were made and spectra in the wavelength of 200-380 nm were obtained (Figure 3).

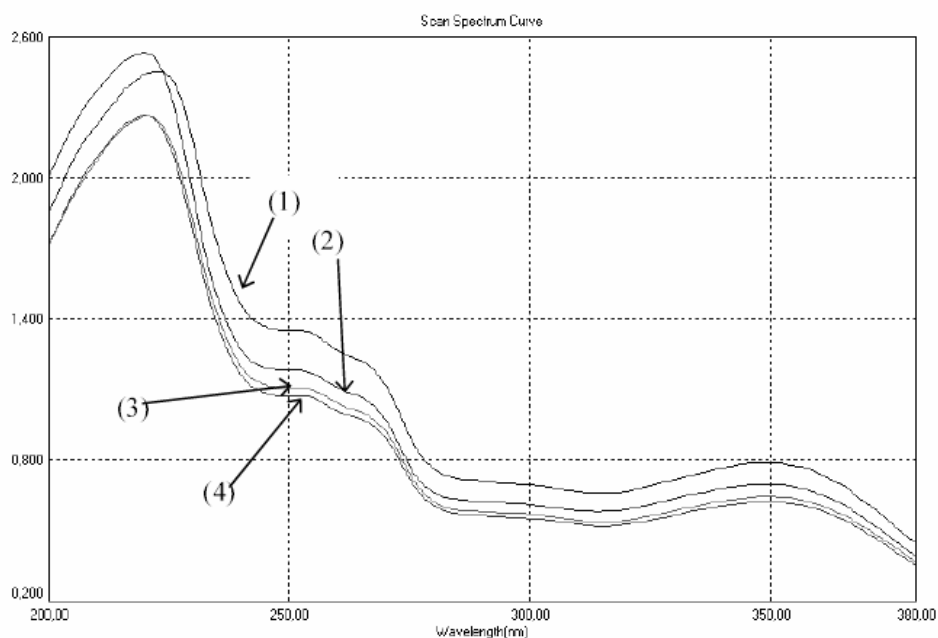


Figure 3. UV spectra of dry sea buckthorn extracts at initial moment (1), after 30 min (2) after 60 min (3) and after 90 min (4)

The spectrum obtained for the extract of white sea buckthorn is complex featuring more absorption peaks. The ascorbic acid absorbs in water at 262 nm while in acetic acid absorbs at 244 nm. In the extract analyzed, there is a peak at 259 nm located between the two values ($244 \div 262$). It also appears an absorption peak, at 274 nm, which is a polyphenolic compound (Robert et al., 2003). The absorption peak, at 350 nm, is given by carotene, a yellow compound present in large quantity in sea buckthorn (Robert et al., 2003).

A worthy point to note is the absorber value at $\lambda_{\max} = 259$ nm: $A = 1.24$ initially and 1.10 after 90 min., which means that the decrease in the extract absorbance in time is smaller in comparison with the solution absorbance of pure ascorbic acid. The AA stability with the extract of sea buckthorn is accounted for by the synergy among the real system components.

Figure 4 shows the spectra of the extract obtained from dried sweet briar at different times. The absorption peak is between 250 nm and 300 nm and is well defined being specific to the ascorbic acid. The stability of the ascorbic acid in the extract of sweet briar is very high in the first 60 min a slight decrease of the absorber being noticeable only after 90 min. This behavior is explained in a similar way with the sea buckthorn, due to the presence of other compounds that contribute to the stability of the chemical equilibrium by synergism.

3.4. Determination of AA from sweet briar and sea buckthorn

Sweet briar and white sea buckthorn are important sources of ascorbic acid and, therefore, they are found in various phito-preparations and functional food. By Tillmans method widely used for AA determination, values of the content of AA were obtained, as given in Table 1. The results are averages of at least three determinations.

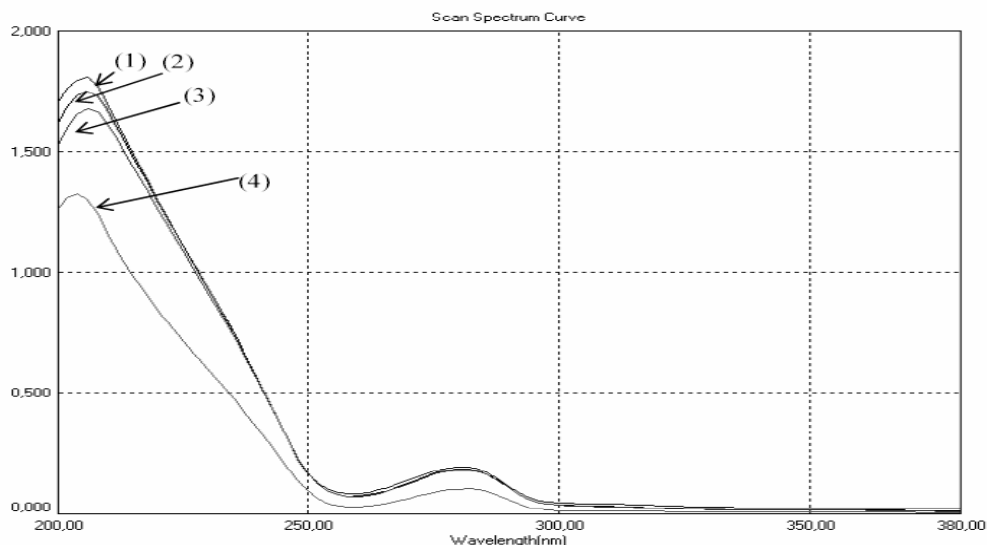


Figure 4. UV spectra of sweet briar extracts dried at the initial moment (1) after 30 min (2), after 60 min (3) and after 90 min (4)

Table 1. Amount (mg) of ascorbic acid from 100 g plant/vegetal product

Sample	Ascorbic acid, [mg/100 g vegetal/plant product]
Dried rosehip	190.004
Dried white sea buckthorn	618.14

From the results obtained, when dosages of ascorbic acid from sweet briar and sea buckthorn are determined by Tillmans method with reagent, the following is found:

- the quantity of ascorbic acid from dried sea buckthorn (618.14 mg/100 g plant product) is a value within the values provided by the literature (400-1500 mg/100g) (Janja and Metka, 2006; Nicholas, 1996);
- the amount of ascorbic acid from dried sweet briar (190.004 mg/100 g plant product) falls within the range of 150-600 mg/100 g, as presented in literature (Janja and Metka, 2006; Nicholas, 1996).

4. Conclusions

The work tried to approach aspects of stability in time of the ascorbic acid solution stored at room temperature and natural light, along with the dynamics of ascorbic acid in solutions with variable pH.

The results were obtained on the modification of the concentration of the two forms taken by the ascorbic acid, depending on the concentration of H_3O^+ ions in the system.

In order to make dosages of the ascorbic acid from plant material, it is very important to maintain the initial concentration of ascorbic acid.

Following the spectrophotometric study on the stability of ascorbic acid in water, trichloroacetic acid and acetic acid, the acetic acid was chosen as solvent extraction of ascorbic acid plant material.

From the quantitative analysis with the Tillmans reagent it has been found that sea buckthorn contains a higher content of ascorbic acid than sweet briar.

The amount of ascorbic acid found from the tests performed is less than that found in the literature (Janja and Metka, 2006; Nicholas, 1996). The result is accounted for by the degree of fruit maturity at harvest. The fruits analyzed in this case, sea buckthorn fruits, were collected in early August. Sea

buckthorn fruits reach optimal ripeness in late September - early October. Sweet briar were collected at the end of September. They reach maturity by the end of October. Also, the amount of ascorbic acid in the fruits of sweet briar and sea buckthorn may be affected by other factors such as the storage conditions.

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