Ascorbic Acid Content in Extractive Aqueous Solutions of *Rosa canina* L. Fruits

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Summary

The main goal of the hereby study is two folded: first, to mark out the most adequate methods of preparing the watery solution extracts (infusions, decoctions) in order to obtain a high content of ascorbic acid, and second, to identify the most suitable method for determining this vitamin in aqueous solution extracts made out of medicinal herbs.

In this experiment six groups were assembled containing 20 fruit samples each. The samples were analyzed one week, one and a half month and three months, respectively, after gathering. Fruit drying was accomplished either in open air, at room temperature, or artificially, for three days, in 15 minutes intervals at 95°C (in the exicator), followed again by room temperature drying.

Preparation of each group was different: it comprised either pickling in cold water for 10 hours, followed by sinking in cold water, boiling and then cooling, or sinking the fruits in boiling water followed by cooling, or sinking the fruits in boiling water followed by boiling the solution for five or 10 minutes, or, finally, by infusion and decoction method.

The results obtained through the Tillmans method revealed a high level of ascorbic acid when the fruits were immersed into boiling water (100°C) and boiled in open fire (11.02 ± 1.51 mg %) for five minutes or when they were introduced in boiling water and kept covered in the boiling basin for 30 minutes (12.26 ± 0.55mg %).

Key words

Cynosbati fructus, ascorbic acid, content, aqueous extract

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Introduction

One of the most encountered vitamins in nature, the ascorbic acid (vitamin C), is considered an *anti-scurvy* and bone-repairing vitamin (Mozaceni, 2002). It intervenes in the calcium fixation on bone (Demir and Özcan, 2001), has an anti-infectious and antitoxic role as it activates the *immune system* of the body (Daels-Rakotoarison et al., 2002; Larsen et al., 2003), it intensifies the detoxifying catabolism of the toxins: nitrites, amines, nicotine, carcinogen substances, and some heavy metals. It is also considered an anti-stress factor (Ciulei et al., 1993; Gheorghe-Mohan, 2000). Through its reducing properties it prevents different biological substances from oxidizing, keeps a reduced number of the thyol groups from glutathyol, and protects the integrity of cell's membrane because it is an antitoxic factor (Crăciun et al., 1988). The ascorbic acid intervenes in a number of metabolic processes such as: the praline and lysine hydrolyzing in the collagen synthesis, (Larsen et al., 2003) it transforms the folic acid in tetrahydrofolic acid and participates in the biosynthesis of the steroid hormones (Nueleanu, 2006; Ognean and Dojană, 2001).

Material and method

The *Rosa canina* L. fruit used in the experiment was harvested at the beginning of September from the spontaneous marchland flora of Cluj-Napoca, Romania. The harvesting grounds were divided into six groups, each containing 20 samples. Each sample contained red fruits with orange hues (Ciulei et al., 1993). These came from a single bush and were harvested in dry and sunny weather.

In order for the fruits to be prepared, the samples were dried-out either in well ventilated places, at room temperature and in the shade or have been heated in a stove for 15 minutes at 95 °C over three consecutive days after which the process continued at room temperature.

The seeds of the dog rose fruits were disregarded as they do not contain ascorbic acid. Only the receptacle was used to prepare 3% watery solution extracts, as recommended in the specialized literature (xxx, 1993). After this the dog rose infusion was prepared form one full teaspoon of fruit in 200-250 ml of water. We observed that one teaspoon of mashed fruits contains between 5.60-5.95 g. Because we only used the receptacle of the fruit, the prepared solution from the whole fruit contains only half of the obtained values.

The groups had been analyzed over three periods of time since harvesting: one week, one month and a half and three months. Only in one case (group II) an analysis over the entire three periods was carried out, as it was considered a control group because this is the most widespread method used in practice (Nueleanu and Ingeborg, 1992).

The fruit samples were divided into six groups according to the preparation mode of the watery solution extracts as following:

- Group I - the fruits (6.0 g) were kept in the cold water for 10 hours to macerate, after which they were immersed into 200 ml of cold water and heated progressively in open fire up to 100°C. The heating lasted for 20-25 minutes and when 100°C were reached the pot was taken and let to cool down.
- Group II – 6.0 g of fruits were put into in 200 ml of boiling water at 100°C, blended and then the produce was let to cool down.
- Group III - the fruits were put directly into boiling water and boiled for five minutes in open fire.
- Group IV - fruits were put directly into boiling water and boiled for 10 minutes in open fire.
- Group V - the infusion method was used: first there were five minutes of preliminary boiling in three parts of cold water, then 200 ml of boiling water at 100°C were added and then everything was let covered at room temperature for 30 minutes (xxx, 1993).
- Group VI - the decoction method was used: first there were five minutes of preliminary boiling in three parts of cold water, then 200 ml of boiling water at 100°C were added followed by boiling in the water basin for 30 minutes under a cover and then everything was let to cool down (xxx, 1993).

In each watery solution extract a determination procedure was carried out next day, without heating the solutions.

The method used in order to determine the ascorbic acid was that of the titrimetric one with 2,6-diclorophenolindophenol (DPI) Tillmans, an often used method in vegetal biochemistry (Andrei and Pintea, 2004).

In order to titrate the diluted solutions prepared for drawing the standard curve, the following quantities of DPI (xxx, 1993) solution were used : 1 mg% = 2 drops; 2 mg% = 3 drops; 5 mg% = 7 drops; 7 mg% = 8 drops; 10 mg% = 11 drops; 20 mg% = 23 drops; 30 mg% = 34 drops.

Results and Discussion

The analyses of the six groups lead to the following results, as mentioned in Table 1. The highest level of ascorbic acid was observed in the fruit from the group I and II one week after harvesting and after immersing in boiling water: 13.20 ± 1.94 mg in the first day and 9.06±1.04 mg in the second day. In the case of the cold water macerated fruit which was in water for 10 hours, the average value decreased over the two days from 8.08±0.83 mg to 6.98±0.65 mg, therefore not so drastically as previous groups. The

Table 1. The average level of the ascorbic acid (mg %) in watery solution extracts from *Rosa canina* L. fruits ($\bar{X} \pm s_{\bar{x}}$)

Groups		I		II		III		IV		V		VI	
Drying method	Fruit age	Day											
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
		Maceration 10 hours		Boiling water		Boiling water boiling 5'		Boiling water boiling 10'		Infusion		Decoction	
95°C (3x15') then room temperature	1 week	8.08 ±0.83	6.98 ±0.65	13.20 ±1.94	9.06 ±1.04								
95°C (3x15') then room temperature	6 weeks			9.31 ±0.85	6.04 ±0.50	11.02 ±1.51	7.58 ±0.88	9.32 ±0.84	6.36 ±0.71				
Room temperature	12 weeks			11.62 ±1.12	5.92 ±0.66					7.52 ±0.78	6.90 ±0.70	12.26 ±0.95	5.94 ±0.30

difference between the two groups was statistically significant ($p<0.05$) only for the first day and not for the second one. On this basis we recommend that the procedures for preparing the watery solutions extracts be made through introducing fruit in boiling water (at 100°C) thus releasing the natural ascorbinase from fruit, which is very active at room temperature (Mozaceni, 2002). The preliminary maceration of the fruit in cold water is not recommended. It is also not recommended to keep the solution for 24 hours at room temperature. In our case such a situation lead to a drastic decrease of the ascorbic acid content by 13.6 % in the case of the solutions prepared through the first method (group I) and by 30.6 % in the case of the second method (group II).

By boiling fruit in water for five minutes and then for 10 minutes (groups III and IV), we could notice that group III had an increased content of ascorbic acid both in the first day with an average of 11.02±1.51 mg and in the second day 7.58±0.88 mg. Average values of ascorbic acid under those of group III, namely 9.32±0.84 mg for the first day and 6.36±0.71mg for the second day could be seen in group IV. The differences between the average values of groups III and IV are insignificant both for the same single day and for consecutive days.

We could also notice that the values of groups IV and II (9.32±0.84 mg and 9.31±0.85 mg) are very close, almost identical, which is indicative of the fact that keeping the fruits for a month and a half, resulted in two equally ineffective methods of solution preparation. Between the average values of groups IV and II the differences were significant only between the two consecutive days of the experiment. In this latter case we could notice a decrease of the ascorbic acid between the two days of the experiment, too. The decrease was by 35.1 % for the group I, with 31.2 % for the group III and by 31.7 % in the case of group IV.

The average value of the ascorbic acid content for the group prepared with the decoction method (group VI) was 12.26±0.95 % on the first day and 5.94±0.30 mg on the second day. The average value for the infused group (V) was 7.52±0.78 mg for the first day and 6.90±0.70 mg for the second day. In our opinion the low values obtained after infusion are due to the fact that the solution was filtered hot, while for the other methods it remained non-filtered until cooling. The level of the ascorbic acid in the case of this method is significantly lower, $p<0.01$.

By applying the "t" test to the average values of groups V and VI we have noticed a significant difference ($p<0.01$) between them. The averages in group V were also significantly different from the control group (II), while the difference between group VI and the control group was not statistically significant ($p<0.01$). The same test applied to the averages from consecutive days within the group was not significant for the infusion, but was highly significant in the case of the decoct ($p<0.001$). This can lead to the conclusion that in the case of the decoct it is not recommendable to keep the fruit for 24 hours, because the vitamin C diminishes considerably.

The "t" test applied to the control group (II) in consecutive days, over a period of three months since the fruits were harvested, leads to the significance of $p <0.001$, very close to that applied to the interval of one month and a half ($p<0.01$). This makes us believe that the content of vitamin C does not decrease if fruits are kept for longer periods.

Dry fruits prepared either through the hot water immersion method (group II) or through the decoct method (group VI) show great loss of ascorbic acid; 49% in the first case and 52.3% in the second case. The loss in the hot filtered infusion was only 8.2%.

Conclusions

The dog rose fruit represent an important source of ascorbic acid as compared to other medicinal herbs, and therefore it is essential to well prepare the watery solution extracts.

We consider that the best way of preparing the fruit is by immersing them into hot boiling water and boiling them in open fire for five minutes. It can be noticed that by

using the decoction method, the level of the ascorbic acid is higher than if compared to all other methods used.

It also recomended to cool the solutions before filtering in order to get higher level of ascorbic acid

It is also advisable that a certain quantity of infusion or decoct be consumed shortly after preparation. If kept for longer than 24 hours the level of ascorbic acid decreases significantly.

It was also noticed that if the decoct method is employed the level of the ascorbic acid is highest. Only a long boiling in open fire destroys vitamin C.

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