Turkish J. Pharm. Sci. 3 (2), 61-72, 2006

EFFECT OF SOME PARAMETERS ON THE EXTRACTION AND DECOMPOSITION OF ASCORBIC ACID IN THE ROSEHIP

Olcay ŞENDİL

Gazi University, Art and Science Faculty, Department of Chemistry,
06500 Ankara, TURKEY

Abstract

The dependence of grain size, solvent volume, temperature and time on the extraction and decomposition of ascorbic acid (AA) in rosehip was studied. Dried rosehip fruits were used as samples after their seeds have been removed, ground, and separated according to different sizes of grains by a sifter. The extracted ascorbic acid (vitamin C) quantity was determined by iodometric method. The results were compared with the results of synthetic ascorbic acid solutions under the same experimental conditions. It was found that the decomposition of synthetic ascorbic acid was faster than ascorbic acid obtained from rosehip. It has been concluded that the amount of ascorbic acid that was extracted doesn't depend on the grain size of rosehip samples. The extraction of ascorbic acid increased depending on the volume of solvent and the temperature. If the same waiting period is taken into consideration, ascorbic acid in the rosehip solution decreases considerably slowly when compared to ascorbic acid in the synthetic solutions.

Key words: *Ascorbic acid; Rosa canina; Rosehip*

Kuşburnundaki Askorbik Asitin Ekstraksiyonu ve Bozunmasına Bazı Parametrelerin Etkisi

Tanecik büyüklüğüne, çözücü hacmine sıcaklık ve zamana bağlı olarak kuşburnundaki askorbik asidin (AA) ekstraksiyonu ve bozunması çalışıldı. Çekirdekleri ayıklanmış, ufalanmış,ve bir elekle farklı boyutlara ayrılmış kuru kuşburnu meyveleri numune olarak kullanıldı. Ekstrakte edilen askorbik asit iyodometrik metotla tayin edildi. Sonuçlar aynı deneysel şartlar altında çalışılan sentetik askorbik asit çözelti sonuçları ile karşılaştırıldı. Sentetik numunelerde askorbik asitin bozunmasının kuşburnundan elde edilen askorbik asitten daha hızlı olduğu bulundu. Ekstrakte olan askorbik asit miktarının kuşburnu örneklerinin tanecik boyutuna bağlı olmadığı tespit edildi. Sıcaklık ve çözücü hacmine bağlı olarak askorbik asit ekstraksiyonu arttı. Bekleme süresi göz önüne alındığında kuşburnu çözeltilerindeki askorbik asit sentetik çözeltilerdeki askorbik asit ile karşılaştırıldığında çok daha yavaş bozunmaktadır.

Anahtar Kelimeler: *Askorbik Asit ; Rosa canina; Kuşburnu*

Corresponding author: olcay@gazi.edu.tr ; Fax: +90 312 212 22 79

INTRODUCTION

Rosehip is a natural plant from the family of Rosaceae that can grow up to 2-6 meters. The inside of the fruit is covered by very thin feathers and there are numbers of seeds. It has a rich composition because of its mineral and vitamin contents. It is used in the food and medicine industry in many countries in Europe. Also, its consumption is considerably common in marmalade, fruit juice, rosehip powder, stewed fruit and tea. Besides, it is used as a herb to cure some ailments such as gum disease, diarrhea, diabetes, stomach and kidney diseases, and hemorrhoids. The rosehips are used in landscaping for esthetic effect and it is also used to prevent erosion. It has many benefits: Its root, trunk, and leaves are used to make pigment and its seeds are used as animal food (1).

The importance of rosehip in matters of health is increased by the fact that the fruit contains more vitamin C, ascorbic acid (AA), (200-5000 mgAA/100 g fresh fruit) than any other commonly available fruit or vegetables. Also, rosehip has a rich composition because of its minerals (K, P, Ca, Mg, Mn) and vitamin contents (C, P, E, B2, B1, provitamin A), (2,3). In addition, rosehip is used in drinks since its soluble dry substance ratio is of high level (20 – 50 %). This is about 11-15% in oranges. pH values change between 3.7-4.4 in its aqueous solutions(4).

Ascorbic acid decomposes easily with the oxygen in air, high temperatures, under sunlight, and in the presence of metal ions like copper in the media that acts as a catalyst for the decomposing reaction of AA and changes it into dehydroascorbic acid (5-8). For these reasons, the decomposition of AA is faster than other vitamins during the storing, cooking, and processing of foods. On the other hand, AA has a good resistance against light and oxygen in dry conditions and at room temperature.

At present, there are many developed instrumental methods (e.g. spectrophotometric (8-15), chromatographic (17,18), polarographic (19,20)) that are highly sensitive and can be used for the determination of AA. On the other hand, the older titrimetric methods are still being used frequently when high concentration of AA is under consideration (21-24). There is no need for fancy and expensive instruments. In this study, ascorbic acid in the rosehip samples has been determined by the iodometric method.

The aim of this work was to determine the ascorbic acid in rosehip tea and to define the best medium for its extraction.

EXPERIMENTAL

Reagents

All reagents were of analytical reagent grade. Distilled water was used for the preparation of all solutions. 0.3 M H_2SO_4 solution was used for acidic medium.

Standard 0.01 M KIO_3 solution was prepared by dissolving 2.000 g of KIO_3 in 1000 ml of water. It was stored in a dark glass bottle.

The 0.07 M $Na_2S_2O_3$ solution was prepared by dissolving 17.4 g of $Na_2S_2O_3$. 5 H_2O in the boiled distilled water that contained 1 g Na_2CO_3 and standardized with standard KIO_3 solution.

The 1 % (m/m) starch solution was prepared by boiling 1 g of soluble starch in about 150 ml of water. It was stored in a glass-stoppered bottle and used for 2 weeks.

Procedures

Solid rosehip samples

The seeds and feathers were removed from the dried fruits of rosehip. They were kept for 1 day at 25 ^{0}C in the drying oven, crumbled by a porcelain mortar and separated into parts of 8, 12, 16, 35, 60, 140 and 400 mesh by a sifter. Because a large of part of the 60 mesh, and almost all of the 140 and 400 mesh became feathers, they weren't studied. The other meshes were stored in the glass-stoppered bottles in the dark. Table 1 shows the mass and percent age of separated parts of the rosehip fruits.

Table 1. Parts of the dried rosehips sample

	mass (g)	% (m/m)
Dried fruit	700	100
Pulp	325	46
Seed	355	51
Feather	20	3

Synthetic ascorbic acid solutions

The solutions were prepared by dissolving a known weight of pure ascorbic acid in 50 ml distilled water. And then they were kept at a fixed temperature (20, 50 and 100 ^{0}C) for different periods of time, cooled to room temperature, and then their ascorbic acid quantities were determined by the iodometric titration methods.

Rosehip solutions

Two types of rosehip solutions were prepared. In the first type of solution, after 30 minutes of extraction of time, the extract was filtered through cotton gauze and then the AA present in the filtrate was determined. The effects of temperature and waiting period on AA concentration in filtrate have been investigated. In the second type of solutions, filtration wasn't made until the titration process, so that the extraction of rosehips was continuous during the waiting period. The effect of temperature and the waiting period on the extraction and decomposition of AA has been studied.

First type of solution: Distilled water (200 ml) was added to the solid rosehip samples of equal weight (2.5 g). The solution was kept at a fixed temperature (20 and 50^{0}C) for 30 minutes (stirring with 5 minutes intervals) and then filtered. The filtrate was kept at the same temperature for different periods of time (30 minutes – 24 hours), cooled to room temperature and then titrated.

Second type of solutions: Equal volumes of water (200 ml) were added to equal amounts of solid rosehip fruit samples (2.5 g) and each solution was kept for different periods of time (30 min. 24 hours) at fixed temperatures (20, 50 and 100 ^{0}C). At the end of the waiting period, the samples were filtered, cooled to room temperature and titrated.

Titration Method

An excess of KI (1-1.5g) and the acid solution (8 ml 0.3 M H_2SO_4) was added to the sample solution (200 ml) then a known volume of standard KIO_3 solution (25.0 ml) was added.

Each mol iodide (IO_3^-) with excess of iodine (I^-) produces 3 mol triiodine I_3^- in the acidic medium. One mole of triiodine reacts with one mole of ascorbic acid and the iodine is reduced while AA is oxidized.

The excess I_3^- was back titrated with standardized thiosulfate solution (0.07 M $Na_2S_2O_3$) and the starch indicator was added towards the end point. The titration was continued until the disappearance of the blue starch-triiodide color.

RESULTS

Effect of solvent volume and the size of grain on the solubility of AA

The solid particulates of rosehip samples (2.5 g) which were extracted 30 minutes in different solvent volumes (distilled water) and then filtered. The filtrate was titrated and its AA content determined. The effect of solvent volume on the extracted quantity of AA is in given Fig. 1. As can be seen extracted AA quantity increased with the increase of solvent volume. Although the experiment was conducted up to 200 ml, no change was observed between 150-200 ml (for 2.5 g rosehip fruit samples). The effect of grain size on extracted quantity of AA (after 30 minutes of extraction) is given in Figure 2. As can be seen there is no connection between the solubility of AA and the size of the fruit grain (8-60 mesh).

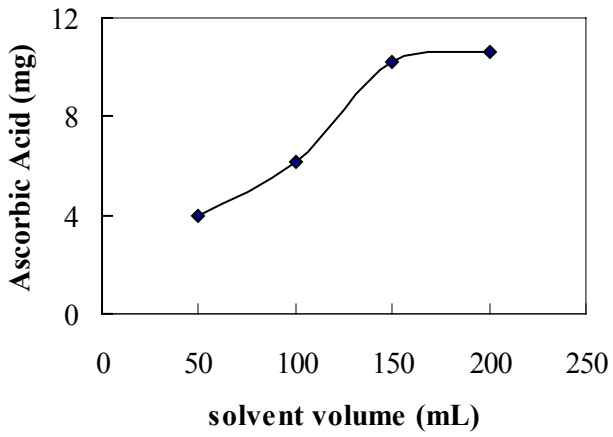

Figure 1. Effect of solvent volume on the extraction of AA in rosehip samples

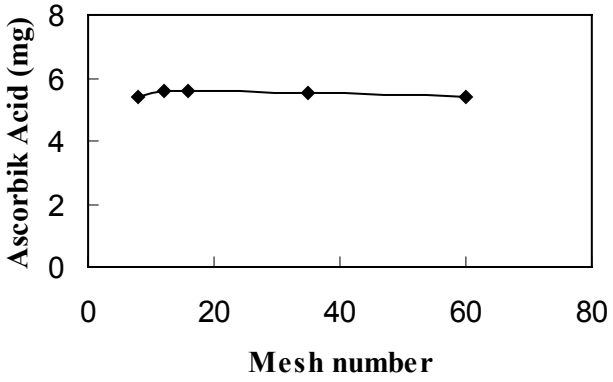

Figure 2. Effect of grain size on the extraction of AA in rosehip samples (solvent volume: 200 ml, 30 min. extraction)

Effect of temperature and time on the extraction and decomposition of AA

1. Synthetic AA solutions

We observed the effects of temperature and time (as shown in Figure 3) on the decomposition of AA in synthetic solution to compare the effect of the substances present in rosehip on the decomposition of AA. The concentration of AA decreased depending with time, and the decomposition of AA increased with the temperature. In 6.5 hours the percentage of decomposed AA was 35, 70 and 90 % at 20, 50 and 100 ^{0}C respectively.

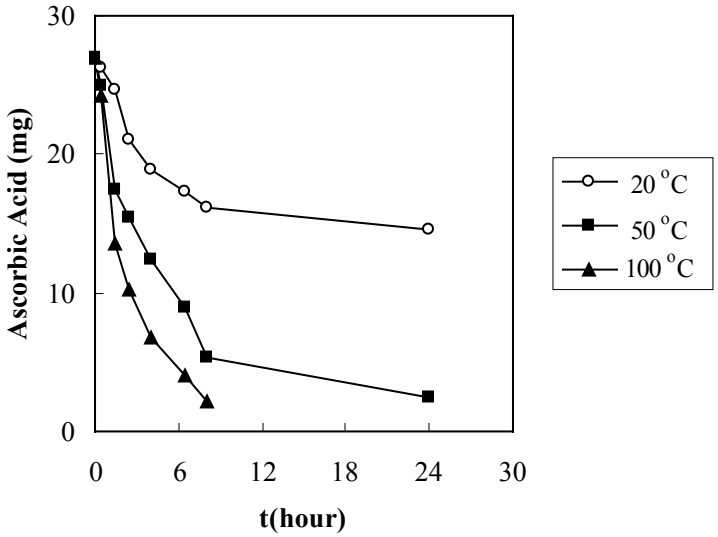

Figure 3. The concentration change of AA in the synthetic samples with time and temperature (Initial concentration of AA: 27 mg).

2. Rosehip solutions

The effect of time and temperature on the extracted decomposition of AA has been studied for two type solutions.

First type of rosehip solution

For this purpose 2.5 g of rosehip in distilled water was filtrated after 30 minutes of waiting period at 20 ^{0}C and 50 ^{0}C. Its AA quantity was determined just after filtration and at 20 ^{0}C the AA was 10 mg, at 50 ^{0}C it was about 15 mg. The filtrate was then analyzed with certain time intervals to observe the decomposition of AA at 20 and 50 ^{0}C. As can be seen from Fig 4 while there was nearly no decomposition in 7 hours at 20 ^{0}C, about 60% was decomposed at 50 ^{0}C. Since the decomposition of the filtrate of rosehip extract was fast at 50 ^{0}C , the decomposition at 100 ^{0}C was not studied. This decomposition rate is still much lower than the synthetic AA under same conditions. These results indicate the existence of some substances in rosehip that protects the decomposition of AA.

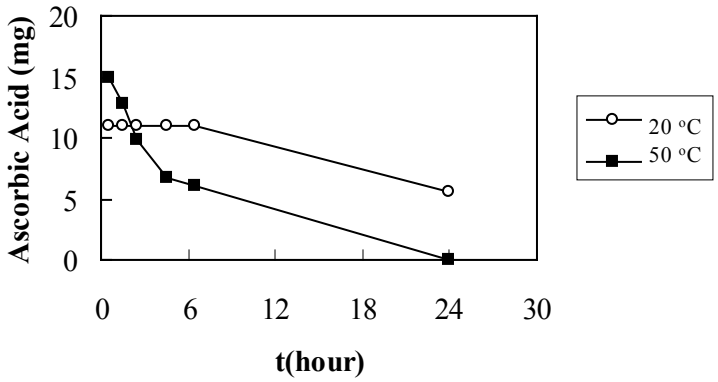

Figure 4. The change of AA concentration in filtrated (first type solutions) rosehip extract by the time at 20 ^{0}C and 50 ^{0}C.

Second type of rosehip solution

Three sets of samples were used. Each set contained 6 samples and each was left wait at certain temperature. First set at 20 ^{0}C, the second at 50 ^{0}C and the third one at 100 ^{0}C. The solution contained rosehip particulates during waiting period. The first measurement for each set was made after 30 minutes. As can be seen from Figure 5, at 20 ^{0}C the AA content was about 10 mg, at 50 ^{0}C about 15 mg and at 100 ^{0}C 35 mg after 30 minute of extraction. The extraction of AA continued at 20 ^{0}C and reached to about 30 mg in 4.5 hours. But then decomposition rate became more effective and AA content decreased to about 15 mg in 24 hours. At 50 ^{0}C the extraction was fast in the first 30 minutes, the AA content reached to 15 mg but it was not so. However, the decomposition rate was faster than the extraction and the AA content decreased. At 100 ^{0}C the extraction rate was fasten it reached to 35 mg and AA content was nearly constant for about 6.5 hours, but then it decomposed faster.

One can conclude that the decomposition of AA is prevented in the rosehip extracts. In the synthetic AA solutions (30 mg) at 50 and 100 ^{0}C in about 6 hours nearly all AA was decomposed. In the first type of solution which was filtered and waited after extraction at 50 ^{0}C in about 6 hours 30% AA was left. In the second type of solutions (still containing small rosehip particulates in waiting period) even at 100 ^{0}C after 6 hours still about 30 mg of AA was present. Thus the maximum amount of AA can be obtained at 100 ^{0}C extraction and this solution may wait about 6 hours without any loss.

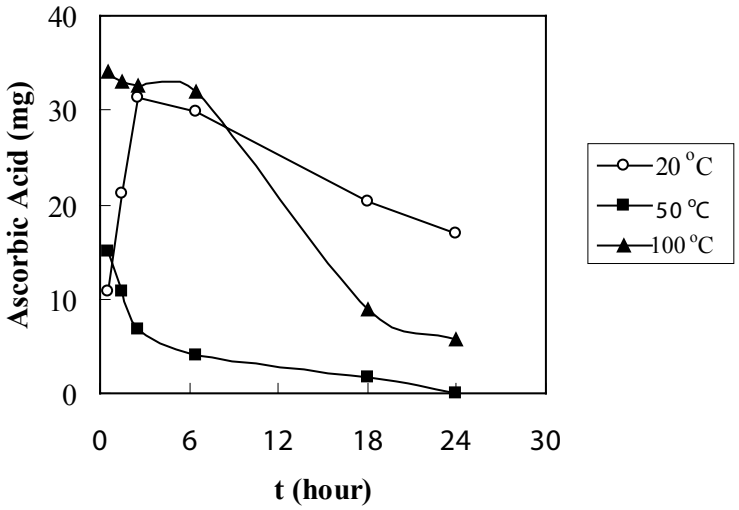

Figure 5. The change of AA concentration in non filtrated (second type solutions) rosehip extract by the time at different temperatures (first data is obtained after 30 minutes of the start).

3. Amount of AA in the same commercial products

The concentration of AA in different brands of rosehip tea and marmalade which were chosen haphazardly were determined and tested at 20 ^{0}C and 100 ^{0}C. The sample solutions were prepared according to the second type of solution (with particulates) and filtrated after a waiting period of 30 minutes and then titrated; the amount of AA that was consumed (hot or cold) was determined. The results are given in Table 2. Also in these samples, the extraction of AA was the highest at 100 ^{0}C. Different values for AA content were found for different brand of tea. This is expected because the growing conditions, storing period, processing of the product were different.

Table 2. Quantities of AA in the some commercial products (30 minutes waiting period for tea bags)

Sample	mg sample	20 ^{0}C			100 ^{0}C		
		mg AA ± SE	% AA	% RSD	mg AA±SE	% AA	% RSD
Gümüşsu bag tea	2.0 x 10^3	6.3 ± 0.5	0.3	17	26.3 ± 1.3	1.3	10
Huge Butlen bag tea	2.0 x 10^3	3.9 ± 0.5	0.2	27	6.6 ± 0.4	0.3	14
Mc Cormick bag tea	2.0 x 10^3	-	-	-	21.4 ± 0.7	1.0	7
Marmalade	15.0x 10^3	-	-	-	8.5 ± 0.5	0.05	13

DISCUSSION

The extracted AA quantity increased with the solvent volume for 2.5 g rosehip sample the maximum amount of extraction was reached with 150 mL solvent volume. There was no effect on extracted quantity of AA with grain size changing between 8-60 mesh.

The decomposition of synthetic AA has been investigated for 24 hours at 20, 50 and 100°C, and it was found that the decomposition is very fast at high temperatures. Similar results were obtained in an investigation where decomposition was studied for 60 minutes (11). It was also shown that AA decomposes in synthetic solution much faster than in the rosehip solutions which contain extracted and filtered, Fig 4. This result was in good agreement with a work where AA decomposition in orange and tomatoes were studied (5,7). These results indicate the existence of some substances in rosehip that protects the decomposition of AA.

The literature survey has shown that there is no work where the decomposition of AA has been studied in the presence of particulates. We also studied the decomposition and extraction of AA solutions containing the particulates of rosehip. According to the results obtained at 20, 50 and 100°C (Fig 5), the extraction rate of AA in the presence of rosehip particulates increases with temperature.

The AA extraction and decomposition degree depends on temperature and time. At 20 °C, extraction is taking place in the first 2-3 hours only and the AA concentration reaches a maximum in about 2.5 hours. On the other hand, decomposition starts on after about 6 hours of waiting period. These results are in good agreement with the results obtained with the filtered solutions, Fig 4. Thus at lower temperatures such as 20 °C, AA will be stable for about 6 hours in its natural medium.

At 50 °C, the decomposition of AA starts on immediately. Although at the beginning the AA concentration was larger than the amount obtained at 20 °C, it decreased with time, which is an indication that the decomposition rate is larger than the extraction rate at this temperature.

At 100 °C on the other hand, the concentration of AA was stable for about 6 hours, an indication that the extraction and decomposition rates are similar at this temperature. Since the extraction rate will be smaller with time one can say that after 6 hours the decomposition rate is larger than the extraction rate.

CONCLUSION

Vitamin C is known as an important vitamin for the health of human beings and it must be taken about 50-75 mg daily by adults (25). If vitamin C is taken from rosehip fruit, non filtrated hot consumption is best. 3-4 bags of tea are enough to satisfy one's daily need of vitamin C. However, products like marmalade that have been boiled and preserved for a long time are not really valuable as far as their content of vitamin C is concerned. Although the maximum amount of extraction was obtained at 100 ^{0}C it should not wait more then 6.5 hours at 100 ^{0}C. If the extracted solution has to be stored longer period of times it can be extracted first at 100 ^{0}C, and than can be stored at temperatures lower than 20 ^{0}C.

REFERENCES

1. **Şen, S.M., Güneş, M.,** The Announcements Book of Rosehip Symposium: Karadeniz Technic University, Gümüşhane/Turkey, September 5-6 , 41-46, 1996.

2. **Demir, F., Özcan, M.**, "Chemical and technological properties of rose (*Rosa canina* L.) grown wild in Turkey" J. Food Eng., 47, 333-336, 2001.

3. **Cai, J.T., Ding, Z.H.**, "Nutrients composition of Rosa laevigata fruits" Science Technology Food Industry, 3, 26-29, 1995.

4. **Yıldız, H., Nergiz, C.,** The Announcements Book of Rosehip Symposium: Karadeniz Technic University, Gümüşhane/Turkey, September 5-6, pp. 309-318, 1996.

5. **Şahbaz, F., Somer G.**, "The effect of citrate anions on the kinetics of cupric ion-catalysed oxidation of ascorbic acid" Food Chem. 47: 345-349, 1993.

6. **Kaanane, A., Kane, D., Labuza, T.P.**, "Time and temperature effect on stability of Morroccan processed orange juice during storage" J. Food Sci. 53(5): 1470-1473, 1990.

7. **Şahbaz, F., Somer G.**, "The stability of ascorbic acid in model systems associated with orange and tomato juices" G.Ü. fen-Edeb. Fak. Fen Bilimleri Dergisi. 4/1: 165-172, 1994.

8. **Birch, G.G., Parker, K.J.**, Vitamin C. London, Applied Science Publishers Ltd, 1974.

9. **Ferreira, S.L.C., Bandeira, M.L.S.F., Lemos, V.A., Santos, H.C., Spinola Costa, A.C., Jesus, D.J.,** "Sensitive spectrophotometric determination of ascorbic acid in fruit juices and pharmaceutical formulations using 2-(5-bromo-2-pyridylazo)-5-diethyllaminophenol (Br-PADAP)" Fresenius J. Anal. Chem. 357: 1174-1178, 1997.

10. **Srividya, K., Balasubramanian, N.,** "Indirect spectrophotometric determination of ascorbic acid in pharmaceutical samples and fruit juices" Analyst. 121: 1653-1655, 1996.

11. **Biurrun, M.C.Y., Romero, R.M.C., Barrera, P.B.**, "Indirect flow injection determination of ascorbic acid by flame atomic absorption spectrometry" Microchim. Acta. 126: 53-58, 1997.

12. **Fung, Y.S., Luk S.F., "**Determination of ascorbic acid in soft drinks and fruit juices (Part 1,2)" Analyst. 201-204, 1439-1444, 1985.

13. **Noroozifar, M., Khorasani-Motlagh, M., Akhavan, K., "**Atomic absorption spectrometry for the automatic indirect determination of ascorbic acid based on the reduction of manganese dioxide" Analytical Sciences 21 (6), 655-659, 2005.

14. **Güçlü, K., Sözgen, K., Tutem, E., Özyürek, M., Apak, R.,** "Spectrophotometric determination of ascorbic acid using copper(II)-neocuproine reagent in beverages and pharmaceuticals" Talanta 65 (5), 1226-1232, 2005.

15. **Zhang, Z.X., Sun, D.M., Rong, Z.H.,** "Catalytic kinetic spectrophotometric determination of trace ascorbic acid" Spectroscopy and Spectral Analysis 24 (7), 873-875, 2004.

16. **Zaporozhets, O.A., Krushinskaya, E.A.,** "Determination of ascorbic acid by molecular spectroscopic techniques" Journal of Analytical Chemistry 57 (4), 286-297, 2002.

17. **Silva F.O.,** "Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography" Food Control 16 (1), 55-58, 2005.

18. **Ziegler S.J., Meier B., Sticher O.**, "Fast and selective assay of *l* – ascorbic acid in rose hips by RP-HPLC coupled with electrochemical and/or spectophotometric detection" Planta Medica. 24: 383-387, 1986.

19. **Mohanadas, C., Indrasenan, P., "**N-Bromosaccharin as an oxidimetric: Direct potentiometric & visual titrations of some typical reductants in aqueous acetic acid medium" Indian Journal of Chem. 26/A: 55-58, 1987.

20. **Şahbas F., Somer G.**, "Determination of ascorbic acid in fruit and vegetables using Normal Polarography" Food Chem. 44: 141-146, 1992.

21. **Hugnes, D.E.**, "Titrimetric determination of ascorbic acid with 2,6-Diclorophenol Indophenol in Commercial liquid Diets" Journal of Pharmaceutical Sciences. 72: 126-129, 1983.

22. **Umesha, K.B., Kumar, K.A., Rai, K.M.L.,** "Iodometric determination of ascorbic acid in bulk and vitamin-C tablets using chloramine-T" Oxidation Communications 25 (4), 566-570, 2002.

23. **Harris, D.C.** Analytical Chemistry. W.H. Freeman and Company, 1982.

24. **Skoog, D.A., West, D.M., Holler, F.J.**, Fundamentals of Analytical Chemistry. Sounders College Publishing, 1996.

25. **Whitney, E.N., Hamilton, E.M.N., Rolfes, S.R.**, Understanding Nutrition. New York: West Publishing Company, pp. 603, 1993.

received: 22.12.2005
accepted: 10.05.2006