

Influence of Traditional Processing on Some Compounds of Rose Hip (*Rosa canina* L.) Fruits Collected From Habitat in Bursa, Turkey

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Twenty seven species of rose hip are grown in different regions of Turkey. It is widely consumed as a tea after drying or processed into marmalade and juice (nectar). In this study, dry matter, lycopene, mineral content (Na, Mg, K, Ca, P), vitamin C, phenolic compounds, antioxidant activity of rose hip fruits grown in Bursa, Turkey and the same contents of theirs products were determined. Some significant changes and loss were fixed after processes. Generally, the highest mineral was Ca and the lowest was Na in fresh rose hip fruits and their products. It is suggested that fresh rose hip fruits and dried rose hips could be used as a source of antioxidant when vitamin C, lycopene and antioxidant activity were evaluated together. It was found that quercetin and (+)-catechin were the main phenolic acids in fresh rose hip fruits and processed rose hip samples.

Key Words: Rose hip, *Rosa canina* L., Phenolics, Lycopene, Minerals, Antioxidant.

INTRODUCTION

There is an increasing global interest towards developing so-called functional foods or finding food additives that would be able to protect human body from diseases caused by oxidative stress in human cells¹. One of these functional foods used for medical purpose is rose hip fruits. The reddish orange colored fruit of rose hip (*Rosa canina* L.) known variously as wild briar, witches briar, dog rose, hip fruit or hip tree is a member of the *Rosaceae* family. Anatolia (Turkey) is located in motherland of rose hips (*Rosa* spp.), which has become widespread around the world. More than 100 species of rose hip have been identified². There are about twenty seven species of rose hip under cultivation in every geographical region of Turkey³.

Rose hip fruits are known as multiple source of natural antioxidants. They are excellent source of vitamin C⁴ and other vitamins as A, P, K, B₁, B₂^{1,5}, vitamin E^{1,6}, some minerals as K, Ca, Na, Fe, Mg and P⁷, folates⁸, carotenoids as α -carotene, lycopene, rubixanthin, gazaniaxanthin, α -cryptoxanthin, zeaxanthin⁹⁻¹¹ and phenolic compounds as quercetin, ellagic acid, quercetin-glycosides, hydroxycinnamic acid, proanthocyanidin aglycones^{1,12,13}. In recent years, an expanded concern about rose

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hips has shown due to its use for different purposes such as a food source and herbal medicine in Turkey. Traditional producers and herbalists have appraised rose hip fruits for the production of tea, dessert, soup, marmalade, purees, fruit juices and dried rose hip fruits. Because of rose hips have biological active compounds, they help building the human body's defense against colds and flu, catarrh, sore throats and chest infections¹⁴. They also reduce symptoms of osteoarthritis¹⁵⁻¹⁷, gastritis, diarrhea, polydipsia⁵ and interestingly, protect the inhibitory capacity against cancer cell proliferation¹³.

In general, orange rose hip fruits are not quite ripe and the reddish-orange fruits of the rose hip (especially, *Rosa canina* L.) are suitable for food processing. Deep red rose hip fruits are overripe. However they are sweet and pulpy, they have lost much of their vitamin C by ripening. In addition, rose hip fruits should be processed immediately after harvesting.

The aim of this study was to determine the biologically important compounds as phenolic acids, minerals, lycopene, vitamin C and antioxidant activity found in fresh rose hip fruits and their products. To find the lose value of these compounds after different processing techniques is the second aim of this research.

EXPERIMENTAL

Caffeic acid (822029), ferulic acid (822070), *p*-coumaric acid (800237), *p*-hydroxy benzoic acid (821814), protocatechuic acid (841533), methanol, hydrochloric acid, oxalic acid, formic acid, acetonitrile were purchased from Merck (Darmstadt, Germany); (+)-catechin hydrate (C-1251), kaempferol (K0133), quercetin hydrate (337951), 2,2-diphenyl-2-picrylhydrazyl radical (DPPH), petroleum ether (analytical grade), 2,6-dichlorophenol indophenol and acetone were purchased from Sigma-Aldrich (St. Louis, USA) while ellagic acid (45140), myricetin (70050) were purchased from Fluka Chemie AG (Buchs, Switzerland).

In this study, reddish-orange rose hip (*Rosa canina* L.) fruits were collected in August 2008 from habitat in Bursa (Aksu, Alacam, Gozede, Gorukle, Yorukali).

Production of rose hip marmalade: In this research, rose hip marmalade was produced from *Rosa canina* L. fruits. In the first step, rose hip fruits were washed and boiled in water by continuous stirring. During the boiling process, hot water is added several times until the end of the boiling process and then rose hip fruits were sifted and sieved with the ear which combines the irritant hairs of seeds. Pulp was passed through tulle and put in a boiler. Half kg sugar was added to per kg rose hip pulp and stirring boiled until bubble up. By not adding any sugar to pulp, sugar free marmalade was produced. After cooking, hot marmalade was put into the jar and then cooled.

Production of rose hip nectar: Rose hip nectar was produced from rose hip puree obtained from a commercial firm established in Bursa, Turkey. The pulp was 6 brix and had a 0.8 % acidity. Nectar was specialized as 40 % fruit ratio, 14 brix and 0.4 % acidity. The product was filled in 200 mL bottles and then pasteurized.

Production of dried rose hips: Rose hip fruits were cut into two halves, seeds were removed and they were well dried naturally under the sun. Finally, they were put into jars with lids closed airtight.

Determination of total dry matter: Due to various water content of samples, all calculations were made according to dry matter basis. For determination of the dry matter content, 3-4 g of homogenized sample (as triplicate) was dried in a hot air oven at 100 ± 5 °C for at least 2 days until reaching constant weight¹⁸.

Determination of ascorbic acid (Vitamin C): Ascorbic acid was determined by using the 2,6-dichlorophenol indophenol (dye solution) spectrophotometric method from the AOAC¹⁹. It was extracted from 10 g samples by homogenizing with 70 mL of oxalic acid. The homogenate was filtered, then used immediately. Firstly, the absorbance of oxalic acid and dye solution mixture was measured with a blank of pure water at 520 nm in 1 cm quartz cuvettes using Shimadzu UV 1208 spectrophotometer. Afterwards the absorbance of filtrate and dye solution mixture was measured with a blank of filtrate and pure water mixture. The results were expressed as mg 100 g⁻¹ by using standard curve.

Determination of lycopene: Lycopene was determined by using spectrophotometric method²⁰. The lycopene was extracted with analytical grade petroleum ether. Firstly, the samples were homogenated and centrifuged at 3000 rpm after adding 5 mL of acetone. Then the supernatant was added to petroleum ether and pure water mixture in separating funnel. The residue was extracted several times with 10 mL of acetone and centrifuged until the colorless obtained. The separating funnel was washed with pure water with 2-3 times (*ca.* 75 mL). Finally, petroleum ether phase was taken to balloon joje and brought to a final volume of 100 mL by adding petroleum ether. The absorbance of extracts was measured at 505 nm in 1 cm quartz cuvettes with a blank of petroleum ether at Shimadzu UV 1208 spectrophotometer. Then the results were calculated and expressed as mg kg⁻¹.

Determination of mineral contents: About 0.5 g homogenized samples were put into a teflon burning cup and added 6 mL pure HNO₃ and 1 mL H₂O₂. The samples were incinerated in a milostone microwave oven and incinerated samples were diluted to 25 mL with distilled water. Then, mineral matters (Na, Mg, K, Ca, P) were analyzed with Inductively Coupled Plasma Optic Emission Spectroscopy (ICP-OES) method²¹.

Determination of antioxidant activity: Antioxidant activity of rose hip and its products was determined by the 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method²² with some modifications. About 1 g samples were extracted with 80 % aqueous methanol (4.5 mL) on a mechanical shaker for 2 h. The mixture was centrifuged at 10,000 rpm for 15 min and the supernatant decanted into polypropylene tubes. The pellets were extracted under identical conditions. Supernatants were combined, filtered and the clear extracts were analyzed for antioxidant activity.

An aliquot of 1.5 mL of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 mL of sample extracts. Instead of methanolic extract of samples, pure

methanol was used as control. The reaction mixture was vortex mixed and let to stand at room temperature in the dark for 1 h before the decrease in absorbance at 517 nm was measured. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation;

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Determination of phenolic compounds: Methanol extraction method was applied with some modifications²³. Approximately 5 g of fresh and dried fruits and marmalade and nectar were extracted with 50 mL of methanol containing 80 mg of ascorbic acid as an antioxidant. 10 mL of 6 M HCl was added to each extract to give a 50 mL solution of 1.2 M HCl in methanol. Samples were extracted at room temperature for 16 h in dark under magnetic stirring. After 16 h, the mixture was filtered. A 15 mL portion of extract was evaporated at 30 °C until 3.5 mL left using rotary evaporator. The extract was filtered through a 0.45 µm filter prior to 10 µL injection to LC-MS/MS.

Working conditions of LC-MS: LC-MS analysis was performed with an Agilent 1100 LC-MSD Trap SL Model LC-MS/MS system equipped with an autosampler. Chromatographic separations were carried out using a Zorbax C18 (50 mm × 4.16 mm i.d, 1.8 µm) column. Mobile phase consists of 1 % formic acid in water (solvent A) and acetonitrile (solvent B). Gradient conditions are as follows; 0-7 min, 30 % B, 7-27 min 50 % B, 27-30 min 33 % B, total run time is 30 min. The column was equilibrated for 10 min prior to each analysis. Flow rate was 0.2 mL/min and injection volume was 10 µL. The mass spectra were recorded in the range of m/z 100-500. Nitrogen was used both as drying gas and as nebulizing gas at flow rates of 5.00 L min⁻¹ and 15.00 psi, respectively. The HV capillary was held at 4000 V. The mass spectra of each compound were obtained at extracted ion mode [M + H]⁺ as illustrated in Table-1. Peaks were identified on the basis of comparison of retention times and MS spectra with standards of ferulic acid, caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid, ellagic acid, (+)-catechin, kaempferol, quercetin and myricetin (Table-2).

TABLE-1
LC-MS CONDITIONS

Time (min)	LC-MS conditions
0	70 % formic acid (1 %), 30 % acetonitrile
7	70 % formic acid (1 %), 30 % acetonitrile
8	50 % formic acid (1 %), 50 % acetonitrile
27	50 % formic acid (1 %), 50 % acetonitrile
27.10-30.00	70 % formic acid (1 %), 30 % acetonitrile

Statistical analysis: The experiment was conducted in a completely randomized design with three replications. The results were statistically evaluated by one way analysis of variance (ANOVA) using the JMP software package version 7.0 (SAS

TABLE-2
RETENTION TIMES (t_R), MOLECULAR WEIGHTS (MW) AND FRAGMENT IONS OF
STANDARD PHENOLIC COMPOUNDS DETERMINED IN THIS STUDY

Phenolic compounds	t_R (min)	MW	[M + H] ⁺	Fragment ions
Ellagic acid	3.40	302	303	259, 279
Ferulic acid	4.60	194	195	177
Caffeic acid	3.40	180	181	163
<i>p</i> -Coumaric acid	4.30	164	165	147
<i>p</i> -Hydroxybenzoic acid	3.60	138	139	121
Protocatechuic acid	3.20	154	155	nd
Quercetin	9.30	302	303	nd
Kaempferol	15.4	286	287	nd
Myricetin	5.10	318	319	nd
(+)-Catechin	3.10	290	291	nd

nd: not detected.

Institute Inc. NC, 27513). The significance of treatments were determined at the 0.05 and 0.01 probability levels, by the F-test. The F- protected least significant difference (LSD) was calculated at the 0.05 probability level according to Steel and Torrie²⁴.

RESULTS AND DISCUSSION

Results of some chemical analysis and the mineral matter contents conducted on fresh rose hip (*Rosa canina* L.) fruits which had been processed into rose hip marmalade, light rose hip marmalade (sugar free), dried rose hip and nectar are indicated in Table-3 and the amounts of the phenolic compounds in Table-4. It is seen that there are significant differences between traditional treatments. These differences were positive or negative depending on the content of the dry matter changing according to the technological processes and the characteristics of the processed product. While fresh rose hip (*Rosa canina* L.) fruits have 34.60 % total dry matter content, it was 81.90 % in dried rose hip, 66.08 % in rose hip marmalade, 13.37 % in light rose hip marmalade (sugar free) and 15.92 % in nectar.

The differences in total dry matter contents arose due to the characteristics of the processed products. In general, dry matter content of the rose hip fruit was found to be higher than the values specified by Demir and Ozcan²⁵ (20.50-23.47 %). These differences could be the results of growing conditions, environmental factors, maturing of the plant and fruit size.

In this study, technological processes caused significant loss in vitamin C content, which was 46.81 mg 100 g⁻¹ in fresh rose hip fruits. While the vitamin C content loss was the highest in dried rose hip (74.27 %), it was followed by nectar (71.25 %), rose hip marmalade (62.92 %), rose hip marmalade (sugar free) (37.96 %) (Table-3). The amount of vitamin C in the rose hip fruits recorded in this study was much lower than previously reported results²⁶⁻²⁸ (140 to 1100 mg 100 mL⁻¹ and 2365 to 2712 mg 100 mL⁻¹ by Demir and Ozcan²⁵). Also, ascorbic acid contents of

TABLE-3
TOTAL DRY MATTER, VITAMIN C, LYCOPENE AND MINERAL CONTENTS OF FRESH AND
PROCESSED ROSE HIP FRUIT SAMPLES

Processed rose hip fruits	Total dry matter (g/100 g)	Vit. C (mg/100 g)	Lycopene (mg/kg)	Na (ppm)	Mg (ppm)	K (ppm)	Ca (ppm)	P (ppm)	Antioxidant activity (%)
Fresh rose hip fruit	34.60 c	46.81 a	75.14 b	161.13 b	868.90 b	1377.23 a	3433.33 b	469.70 b	90.58 a
Dried rose hip	81.90 a	12.04 e	263.16 a	917.93 a	2233.66 a	1308.27 b	4827.00 a	1201.67 a	36.53 e
Rose hip marmalade	66.08 b	17.36 c	20.10 d	122.57 b	242.63 d	585.33 d	905.80 cd	212.77 d	51.78 d
Rose hip marmalade (Sugar free)	13.37 e	29.04 b	54.07 c	133.00 b	385.10 c	1206.87 c	1536.60 c	289.70 c	85.36 b
Nectar	15.92 d	13.46 d	17.56 d	155.75 b	76.83 e	229.43 e	240.43 d	151.77 d	61.64 c
LSD (p < 0.05)	0.85	5.05	8.94	193.29	70.23	68.47	710.41	62.18	2.24

LSD (Least Significant Difference) values with different letters in the same column indicate significantly different at p < 0.05.

TABLE-4
SOME PHENOLIC COMPOUNDS OF FRESH AND PROCESSED ROSE HIP FRUIT SAMPLES (mg kg⁻¹ FRUIT)

Processed rose hip fruits	EA	FA	CA	<i>p</i> -CA	<i>p</i> -HBA	PA	QU	KAM	MYR	(+)-CAT
Fresh rose hip fruit	nd	nd	nd	nd	nd	nd	2.76 b	nd	nd	14.19 a
Dried rose hip	nd	nd	nd	nd	nd	nd	5.20 a	nd	nd	3.48b
Rose hip marmalade	nd	nd	nd	nd	nd	nd	0.00 c	nd	nd	0.00c
Rose hip marmalade (Sugar free)	nd	nd	nd	nd	nd	nd	0.23 c	nd	nd	4.55 b
Nectar	nd	nd	nd	nd	nd	nd	0,00 c	nd	nd	0.14c
LSD (p < 0.05)	–	–	–	–	–	–	0.39	–	–	1.10

LSD (Least Significant Difference) values with different letters in the same column indicate significantly different at p < 0.05.

EA: Ellagic acid; FA: Ferulic acid; CA: Caffeic acid; *p*-CA: *p*-Coumaric acid; *p*-HBA: *p*-Hydroxybenzoic acid; PA: Protocatechuic acid; QU: Quercetin; KAM: Kaempferol; MYR: Myricetin; (+)-CAT: (+)-Catechin; nd: not detected.

some small fruits, including blackberry, raspberry, currant, blueberry, strawberry and gooseberry, ranged from 10.58 to 939 mg kg⁻¹ according to different literatures^{23,29-33}. Ascorbic acid contents of rose hip are higher than most of these small fruits.

The content of vitamin C is affected by genetic factors as well as ecological, climatic and edaphic influences. It has been observed that long rainy periods during the growing season decreases the amount of vitamin C. Plants growing at higher altitudes receive greater light intensities which increases the amount of vitamin C in fruit. In addition, lack of phosphorus or an excess of potassium in the soil decreases the amount of vitamin C. Basar³⁴ also stated that the soil in the Bursa region is of this characteristic. These factors may contribute to explaining the reason that the rose hip fruits in this region are not rich in vitamin C. As known, thermal damage directly relates with the temperature and time. The high temperature and long drying time associated with conventional hot air drying often causes heat damage and adversely affects texture, color, flavor and nutritional value of products. Degradation of ascorbic acid depends on several factors, which include oxygen, metal ion catalysis, light, temperature and moisture contents³⁵.

In this studies, maximum loss of vitamin C was shown in dried products (Table-3). Any pre-oxidation process for preventing vitamin C loss was not done. Also high temperature and long time of drying with oxygen existence caused a loss. Similar results with the air temperature during application and production are the themes that have been seen in other products. Erentürk *et al.*³⁵ reported that the degradation of vitamin C could be reduced by using an inert gas. According to the European pharmacopoeia, rose hip is a big source of vitamin C (at least 500 mg 100 g⁻¹ fruit powder) that shows anti-inflammatory properties and clinically reduces osteoarthritis symptoms³⁶. According to the findings obtained from this study, in order for the rose hip fruits and rose hip products produced with traditional methods in Turkey to provide the aforementioned benefits, they need to be enriched with addition of ascorbic acid.

The lycopene content was measured as 75.14 mg kg⁻¹ at the fresh rose hip fruits, except dried rose hip (263.16 mg kg⁻¹). This content was found to be significantly low in other treatments [rose hip marmalade (sugar free) (54.07 mg kg⁻¹), rose hip marmalade (20.10 mg kg⁻¹), nectar (17.56 mg kg⁻¹)] (Table-3). The increase in dried rose hip fruits was proportional and dependent upon the increase in dry matter content. As it is known, carotenoids, which also include the lycopenes, are pigments that are very susceptible to light, pH, a_w, SO₂, metal ions, high temperatures and oxygen during processing. Degradation is shown and loss of color is observed in products when they are exposed to high temperatures for extended periods of time³⁷. It is considered that the reduction of lycopene content in processed rose hip samples such as rose hip marmalade and nectar is a result of oxidative degradation during processing. The dry matter was consisted of only fruit components in sugar free marmalades, so the lycopene value was higher than in proportion to

others. As similar, the same aspect was seen in other analysis results (Table-3). Bohm *et al.*³⁸ reported the content of total lycopene in raw rose hips ranged from 12.9 to 35.2 mg 100 g⁻¹ and in rose hip products between concentrations of 2.3-5.2 mg 100 g⁻¹. On the other hand, Cemeroglu *et al.*³⁹ stated that the lycopene content in preserved rose hip samples is 0.78 mg 100 g⁻¹. Also taking into consideration the regional differences, the results of this study show similarities with those of the other researchers. Hodisan *et al.*⁴⁰ reported that the main identified carotenoids in rose hip were β -carotene (20.8 %), lycopene (27.8 %), rubixanthin (23.5 %), β -cryptoxanthin and zeaxanthin with lutein (11.3 %). It is reported⁴¹ that a daily intake level of 5-7 mg of lycopene in normal healthy humans may be sufficient to combat oxidative stress and prevent chronic diseases. It was seen that the lycopene content of fresh rose hip fruits was almost similar to lycopene content of tomato (7.85 mg 100 g⁻¹), indicated by a number of researchers³⁷ and considered to be a major source of lycopene. Consequently, fresh rose hip fruits and rose hip products can be a good alternative for those people who do not eat tomatoes and tomato products for protecting them against certain health risks.

While the antioxidant activity was determined to be 90.58 % at the fresh rose hip fruits, this content was found to be significantly low in other treatments (Table-1). The lowest antioxidant activity was measured in the dried rose hip fruits (36.53 %) that were dried naturally under the sun. In other processed products containing fruit ratio based on the proportional change was monitored. Phenolic matters also have a high antioxidant activity⁴¹, so there is a linear correlation between total phenolic content (Tables-3 and 4) and antioxidant activity of rose hip fruits and their products. On the other hand, when studied the antioxidant capacity of individual phenolics in different combinations found in fruits and vegetables, such as catechin, quercetin 3-glucoside, chlorogenic acid, *etc.*, the researchers found only a modest synergistic effect among the polyphenolics⁴².

The mineral contents of rose hip fruits and products are shown in Table-4. Sodium, Mg, K and Ca contents of rose hip fruit were higher, except P, according to findings of Demir and Ozcan²⁵. Also, K contents of fruit samples were found lower than the results (4200-11900 ppm) reported by Ercisli²⁶ and Kovacs *et al.*⁴³. On the other hand, Ca and Mg values were similar to those of same researchers. These differences could be the result of some factors as ecology, growth conditions and composition of the soil, especially. Nonetheless, P, Ca, Mg and Na contents of all analyzed samples were higher than some small fruits such as strawberry, redcurrant, blackcurrant, raspberry, blueberry and blackberry⁴¹.

Polyphenolic compounds are important fruit constituents, by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxyradicals⁴². Phenolics also play an important role as aroma compounds and provide astringency to food products. Phenolic compounds in rose hip fruits and products are given in Table-4. Quercetin and (+)-catechin were determined in methanol extracts of rose hip fruits and products. But, ferulic acid, caffeic acid, *p*-coumaric acid, *p*-

hydroxybenzoic acid, protocatechuic acid, kaempferol and myricetin were not determined. Researchers stated that the major phenolic compounds found in rose hip are hydroxycinnamic acid, catechin, quercetin and kaempferol³³. Olsson *et al.*¹³ also reported that benzoic acid and ellagic acid can not be seen in rose hip fruits.

Conclusion

When the results were generally evaluated, rose hip fruit and its products were considered to be rich of vitamin C, lycopene and minerals (Na, Mg, K, Ca, *etc.*). As phenolic compounds, vitamin C and antioxidant activity were evaluated together, especially fresh fruit and dried samples can be used as antioxidant source. However, some of the components get a loss during processing, rose hip products should be in human diet because of high nutritional value and therapeutic features of rose hip.

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