

Phytochemical Changes in Heated *Rosa* Species Fruits and Seeds

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Abstract

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The phytochemical changes in heat-treated *Rosa* species fruits and seeds were determined. Fruits and seeds of five advanced genotypes belonging to *Rosa dumalis*, *R. canina*, and *R. villosa* were used. Fruits were harvested at optimal maturity and boiled in distilled water for 30 min, followed by analysis of soluble solid content, dry matter, pH, titratable acidity, total sugar, β -carotene, α -tocopherol, and vitamin C. Total phenolic content and the antioxidant activities of fresh and boiled fruits were also analyzed, and fatty acid levels in fresh and heated seeds were determined. Total sugar, pH, and antioxidant activities were not affected, but β -carotene was affected significantly by heating in all species. The losses in β -carotene, α -tocopherol, and vitamin C were between 78–86, 29–51, and 12–60%, respectively. Heat treatment did not affect significantly the ratios of major fatty acids except for *R. dumalis* (MR-12 and MR-46).

Keywords: α -tocopherol; β -carotene; vitamin C; fatty acids; phenolics

Worldwide, the fruits of many rosehip species are collected and processed for various purposes such as marmalade, jam, tea and others. Rosehip products have been restricted to certain regions of the world until recent years, but are now used for many purposes, e.g., as a functional food, and for cosmetic and medicinal applications.

The application of heat treatment to fruit accelerates the disintegration of the cell membrane and the release of phytochemicals from chloroplasts. It also facilitates their easy extraction. In case the cell membrane does not disintegrate, heat treatment promotes the transition of cell fluid to the outer environment (HOWARD *et al.* 1999; LEONG & OEY 2012). It is thought that considerable changes occur in the phytochemical content of fruits when these are subjected to heat treatment. Cooking of long duration leads to thermal destruction of the phytochemicals

which affect antioxidant activity (GRAJEK & OLEJNIK 2010; LEONG & OEY 2012). However, a medium degree ($\leq 100^\circ\text{C}$) of boiling has less harmful or even helpful effects on phytochemicals. Water extraction one hour after the boiling point has been reached produces the extract with the highest antioxidant activity (ANGELOV *et al.* 2014).

Important studies have been carried out on many fruit and vegetable species (except rosehips) concerning the phytochemical changes that occur as a result of heat treatment. It is important to develop methods devoted to the preservation of high vitamin C, β -carotene and α -tocopherol levels, or methods that prevent phytochemical loss.

Rosehip fruits are boiled for a long duration for the production of marmalade or for the infusion of tea by traditional methods. The literature describing the phytochemical changes that occur during the

heat treatment of rosehip products is limited, and, therefore, it remains unclear what kinds of changes take place in phytochemicals or other constituents under these conditions. Thus, the aim of this study was to describe the phytochemical and antioxidant activity changes occurring in heat-treated fruits and seeds of advanced *Rosa* species genotypes.

MATERIAL AND METHODS

Plant materials. Fruits of *Rosa dumalis* (genotype MR-12 and MR-15), *R. canina* (MR-26), *R. dumalis* ssp. *boissieri* (MR-46) and *R. villosa* (MR-84) planted in the research and application area of Agricultural Faculty of Gaziosmanpasa University were used as plant material. The fruits were harvested at optimal harvest time.

Instruments and chemicals. A HPLC-DAD (Shimadzu, Japan) system was coupled with an LC 20AT pump and a SPD-M20A model DAD detector. A Hitachi U-2900 spectrophotometer (Japan) and an FID gas chromatograph (Perkin Elmer Clarus 500 series) were used. HPLC-grade β -carotene and α -tocopherol were purchased from Sigma-Aldrich (Germany). HPLC-grade methanol, acetonitrile and formic acid were purchased from Merck (Turkey). Supelco 37 component FAME Mix, 47885-U was used as standard for quantitative fatty acids analyses by GC-FID.

Titrateable acidity, dry matter, soluble solid content and pH assessment. The soluble solid content (SSC) was determined using a digital refractometer and expressed as Brix in juice. The pH was determined using a pH meter. The titrateable acidity (as citric acid) was measured in 10 g fruit flesh (hypanthium) homogenised in 50 ml distilled water and titrated with 0.1 M NaOH with an end-point of pH 8.1. The weight of dry matter was determined after weighing the hypanthium and drying at 85°C.

Total sugar. Fruit samples (25 g) were disintegrated with liquid nitrogen and extracted with 250 ml of 80% ethanol. Supernatant was taken to a fresh beaker after centrifugation and the same procedure was repeated on the remaining pulp. The supernatant was used for determination of soluble sugar content. The remaining pulp was incubated in boiling water for 30 min after the addition of 5 ml hydrochloric acid (1.1%). These samples were used for the analysis of centrifuged sugar and water insoluble sugar. After preparation, these samples were diluted at the ratio of 1 : 10, and 1 ml was extracted and combined with

3 ml anthrone/sulphuric acid solution (1 mg/ml). The samples were vortexed at room temperature and read at 620 nm wavelength on a spectrophotometer. Glucose was used as a standard. The results were expressed as percentage using an external standard calibration (FERMANIA *et al.* 1995).

Vitamin C. Vitamin C content was determined according to the spectrophotometric method of KHAN *et al.* (2006). The results were calculated as mg/100 g using an external standard calibration which was prepared with ascorbic acid.

β -carotene and α -tocopherol. The modified extraction method developed by KAZAZ *et al.* (2009) was used for the determination of α -tocopherol. Fruit (25 g) samples were disintegrated with liquid nitrogen. Fruit sample powder was extracted with *n*-hexane and the solvent was removed. It was extracted again with ethyl alcohol before HPLC analyses and injected into the HPLC after microfiltration (0.22 μ m). Thermo Scientific Synchronis C8 LC columns (150 $\times$ 4.6 mm, 3 μ M) was used with methanol with a flow rate of 1 ml/min. The amounts of α -tocopherol and β -carotene in the samples were calculated using the calibration curve constructed using the standards and the results were given as mg/100 g.

Total phenolic compound analysis. Total phenolic compounds were determined using a spectrophotometric method with Folin-Ciocalteu reagent after extraction in accordance with the method of SLINKARD and SINGLETON (1977). The amount of total phenolic compounds was calculated as phenolic substance equivalent to 1 mg gallic acid in 100 g fruit using the calibration curve.

Antioxidant activity tests. FRAP analysis was used to determine antioxidant activity in a spectrophotometric method developed by BENZIE and STRAIN (1999). Activity was calculated using a trolox calibration curve used as a standard and the results were expressed as g of fresh fruit equivalent/ μ mol of trolox. As a further method, TEAC analysis was used, according to the method applied by UGGLA (2004). Results were calculated as for FRAP analysis and were expressed as μ mol of trolox/g fresh fruit.

Fatty acids. The fats were extracted using cold extraction (TURKEKUL *et al.* 2006). A mixture of standard fatty acids was used to enable qualitative and quantitative analysis.

Preparation of samples for heat treatment. Sample size was the same as for non heat-treated samples. After the addition of a double volume of distilled water (800 μ l distilled water for 400 μ g sample),

samples were homogenised by vortexing in the test tube. This homogenous mixture was incubated in 100°C boiling water for 30 min and then analysed.

Statistical Analysis. The three replicates were subjected to an analysis of variance (SSPS v15.0). $P < 0.05$ was considered significant. The differences between the means were determined by using Duncan's multiple range test.

RESULTS AND DISCUSSION

The data of fresh and heat-treated *Rosa* species fruits are summarised in Table 1. Total sugar, pH, and antioxidant activities of the studied species were not affected, but β -carotene levels were significantly affected by heat treatment. Further, SSC of *R. dumalis* (MR-12), titratable acidity of *R. dumalis* (MR-46) and *R. villosa* (MR-84), dry matter of *R. dumalis* (MR-12 and MR-15), total phenolic compounds of *R. dumalis* (MR-46), α -tocopherol of *R. dumalis* (MR-15 and MR-46), and vitamin C of *R. dumalis* (MR-12 and MR-46) were affected significantly. The percentage losses of β -carotene, α -tocopherol, and vitamin C varied between 78–86, 29–51, and 12–60%, respectively (Table 1).

YILDIZ and ALPASLAN (2012) reported significant decreases in ascorbic acid, total phenolic compounds and antioxidant activity but increases in anthocyanin and β -carotene up to 100%. In a study that investigated the effects of processing methods, the vitamin C content of the product decreased significantly (ROPCIUC & LEAHU 2014). In another research in which the effects of different processing methods on phytochemicals in fruits and vegetables were investigated, it was determined that both increases and decreases in the levels of anthocyanin compounds occurred as a result of thermal processing. Levels of reducing sugar decreased, those of β -carotene and lycopene decreased or increased and those of vitamin C increased or did not show any change (LEONG & OEY 2012). PAUL and GHOSH (2012) reported that the loss of ascorbic acid and phenolic content in pomegranate juice increased with temperature and the duration of processing. Also, heat treatment resulted in decreases in all physico-chemical parameters, such as SSC, pH, total acidity of juice, with the exception of reducing sugar. In another study, it was reported that the thermal processing of 50, 100, and 150°C/60 min increased the antioxidant activity in citrus rind (JEONG *et al.* 2004). Apart from heat,

also pH and O₂ levels, and other phytochemicals in the environment may act to decrease the levels of flavonoids (IOANNOU *et al.* 2012). ARANCIBIA-AVILA *et al.* (2012) determined that there was no significant differences between samples subjected to thermal processing at 100°C for 10 and 20 min, and those which were not processed, and there was a high correlation between the total phenols, flavonols and antioxidant activity. Thermally processed berries did not maintain their bioactivities for more than 20 minutes. Lycopene and β -carotene bioavailability increased during the processes of mechanical fragmentation and heat treatment, but other substances that negatively affect causing antioxidant activity could not be detected. The increase in carotenoids was correlated with enzymatic deterioration, and with a weakening of protein-carotenoid stacks and density of dry matter caused by evaporation (VAN BOEKEL *et al.* 2010). CAPANOGLU *et al.* (2008) observed significant decreases both in β -carotene and lycopene during the preparation of tomato paste. ARANCIBIA-AVILA *et al.* (2012) highlighted the fact that most investigators have found that the thermal processing of fruits and vegetables decreases the content of bioactive compounds and of antioxidant activity. The total phenolic content of fruit juice prepared at 130°C for 3 h was 2.67 mg GAE/ml, compared to 0.35 mg GAE/ml for juice prepared at 100°C for 1 h (LEE & LEE 2012).

Some of our data are in accordance with previous results, while our results with respect to vitamin levels exhibit some differences: some were lower than in previous studies and some were found to be higher. Levels of vitamin C, β -carotene, and α -tocopherol were more negatively affected by thermal processing. In contrast to previous reports (JEONG *et al.* 2004; LEE & LEE 2012; ANGELOV *et al.* 2014), antioxidant activities were not affected significantly and were not affected by changes in vitamin C levels. Besides vitamin C, it is known that the levels of total phenolic compounds are also associated with antioxidant activity (ELMASTAŞ *et al.* 2015). This can be explained by the fact that the levels of total phenolic compounds are not influenced by heat treatment.

Fatty acids. The percentages of linoleic, oleic and linolenic acids varied between 39–53, 13–36, and 15–24%, respectively. Heat treatment did not affect the oleic acid ratio of any species, but significantly affected the ratios of linoleic acid of *R. dumalis* spp. *boissieri* (MR-46), linolenic acid of *R. dumalis* (MR-12) and *R. dumalis* ssp. *boissieri* (MR-46)

Table 1. Levels of phytochemicals in fresh and heat-treated *Rosa* fruits ($n = 3$)

Species (genotype)	pH			Change (%)			SSC (%)			Change (%)			titratable acidity (%)		
	Fresh	Heated	Change (%)	Fresh	Heated	Change (%)	Fresh	Heated	Change (%)	Fresh	Heated	Change (%)	Fresh	Heated	Change (%)
<i>R. dumalis</i> (MR-12)	3.64 ± 0.11	3.67 ± 0.23	0.82	18.00 ^{bs}	± 1.73	22.33 ^a	± 1.53	24.06	14.24 ± 1.81	14.54 ± 1.95	2.11	2.00 ± 0.16	1.96 ± 0.23	–2.00	
<i>R. dumalis</i> (MR-15)	3.66 ± 0.08	3.77 ± 0.08	3.01	19.67 ± 3.79	20.67 ± 0.58	5.08	12.20 ± 0.72	11.41 ± 6.96	–6.48	2.13 ± 0.23	1.91 ± 0.19	–10.33			
<i>R. canina</i> (MR-26)	3.46 ± 0.11	3.44 ± 0.17	–0.58	22.33 ± 1.53	22.00 ± 2.00	–1.48	11.91 ± 0.75	12.00 ± 0.64	0.76	2.19 ± 0.18	1.95 ± 0.28	–10.96			
<i>R. dumalis</i> ssp. <i>boissieri</i> (MR-46)	3.78 ± 0.15	3.75 ± 0.12	–0.79	24.00 ± 2.00	23.00 ± 1.00	–4.17	12.39 ± 1.53	13.85 ± 4.12	11.78	2.51 ^a ± 0.16	1.87 ^b ± 0.25	–25.50			
<i>R. villosa</i> (MR-84)	3.53 ± 0.17	3.59 ± 0.12	1.70	24.00 ± 1.00	23.00 ± 0.00	–4.17	18.18 ± 2.09	15.91 ± 2.70	–12.49	2.17 ^a ± 0.16	1.87 ^b ± 0.08	–13.82			
Dry matter (%)															
				Total phenolics (mg GAE/100g)				FRAP (μmol trolox equivalent/g)				TEAC (μmol trolox equivalent/g)			
<i>R. dumalis</i> (MR-12)	58.93 ^a	± 2.36	52.84 ^b	± 0.70	–10.33	512.12 ± 10.82	478.04 ± 54.81	–6.65	118.39 ± 15.69	147.87 ± 10.02	24.90	62.19 ± 10.60	74.41 ± 16.73	19.65	
<i>R. dumalis</i> (MR-15)	58.51 ^a	± 2.88	50.43 ^b	± 2.05	–13.81	981.08 ± 24.13	1070.51 ± 74.4	9.12	274.73 ± 37.54	298.88 ± 30.38	8.79	194.56 ± 18.67	201.28 ± 24.20	3.45	
<i>R. canina</i> (MR-26)	45.90 ± 2.29	43.30 ± 1.94	–5.66	596.37 ± 41.12	605.37 ± 63.94	1.51	153.94 ± 7.64	168.60 ± 9.94	9.52	90.00 ± 2.70	89.07 ± 6.70	–1.03			
<i>R. dumalis</i> ssp. <i>boissieri</i> (MR-46)	52.11 ± 2.81	50.94 ± 0.58	–2.25	616.28 ^b ± 3.31	717.16 ^a ± 24.99	16.37	198.03 ± 3.15	194.77 ± 1.07	–1.65	117.91 ± 14.81	128.34 ± 17.96	8.85			
<i>R. villosa</i> (MR-84)	52.64 ± 3.06	52.13 ± 1.33	–0.97	711.43 ± 49.73	816.95 ± 78.55	14.83	205.57 ± 1.80	194.85 ± 10.91	–5.21	135.80 ± 4.61	145.97 ± 15.85	7.49			
β-Carotene (mg/100g)															
				α-Tocopherol (mg/100g)				Total phenolics (mg GAE/100g)							
<i>R. dumalis</i> (MR-12)	1.60 ^a	± 0.73	0.34 ^b	± 0.27	–78.75	1.57 ± 1.05	0.88 ± 0.22	–43.95	285.92 ^a ± 17.3	114.71 ^b ± 31.23	–59.88				
<i>R. dumalis</i> (MR-15)	1.94 ^a	± 0.83	0.34 ^b	± 0.14	–82.47	2.32 ^a ± 0.52	1.18 ^b ± 0.14	–49.14	860.36 ± 9.99	761.15 ± 115.51	–11.53				
<i>R. canina</i> (MR-26)	0.95 ^a	± 0.25	0.21 ^b	± 0.05	–77.89	1.39 ± 0.25	0.99 ± 0.08	–28.78	407.53 ± 28.13	324.32 ± 41.85	–20.42				
<i>R. dumalis</i> ssp. <i>boissieri</i> (MR-46)	2.90 ^a	± 0.45	0.61 ^b	± 0.12	–78.97	2.20 ^a ± 0.37	1.08 ^b ± 0.08	–50.91	341.92 ^a ± 27.3	233.12 ^b ± 37.49	–31.82				
<i>R. villosa</i> (MR-84)	2.27 ^a	± 0.54	0.31 ^b	± 0.19	–86.34	1.69 ± 0.35	1.16 ± 0.67	–31.36	482.73 ± 31.48	373.92 ± 67.43	–22.54				

*differences between fresh and heated averages from the same genotype indicated by different letters in the same line ($P < 0.05$) are significant

Table 2. Levels of fatty acids in fresh and heat-treated *Rosa* seeds (%) ($n = 3$)

	<i>R. dumalis</i> (MR-12)			<i>R. dumalis</i> (MR-15)			<i>R. canina</i> (MR-26)			<i>R. dumalis</i> ssp. <i>boissieri</i> (MR-46)			<i>R. villosa</i> (MR-84)		
	fresh	heated	change (%)	fresh	heated	change (%)	fresh	heated	change (%)	fresh	heated	change (%)	fresh	heated	change (%)
Palmitic	3.59 ^b	4.72 ^a	31.48	3.82	3.97	3.93	4.22	4.31	2.13	3.70	3.35	-9.46	4.48	3.91	-12.72
Stearic	2.91	1.86	-36.08	2.55	2.41	-5.49	3.84	3.91	1.82	3.89 ^b	4.25 ^a	9.25	2.60 ^b	2.92 ^a	12.31
Oleic	35.62	35.47	-0.42	23.56	24.27	3.01	25.64	25.89	0.98	13.39	13.61	1.64	21.60	22.20	2.78
Linoleic	39.23	39.14	-0.23	45.72	45.28	-0.96	46.64	46.02	-1.33	53.13 ^a	52.07 ^b	-2.00	50.64	50.71	0.14
Linolenic	16.13 ^a	14.93 ^b	-7.44	21.30	20.83	-2.21	17.65	18.04	2.21	23.61 ^b	24.47 ^a	3.64	17.49	17.74	1.43
Gama-linolenic	0.93 ^a	0.47 ^b	-49.46	1.06	0.96	-9.43	1.33	1.42	6.77	1.20 ^b	1.62 ^a	35.00	0.67 ^b	1.30 ^a	94.03
Heneicosanoic	0.93 ^a	0.30 ^b	-67.74	0.47	0.42	-10.64	0.36 ^b	0.45 ^a	25.00	0.21	0.21	0.00	0.00	0.00	0.00
Arashidic	0.09 ^b	0.73 ^a	711.11	0.13	0.20	53.85	0.02 ^a	0.00 ^b	-100.00	0.04	0.00	-100.00	0.20	0.00	-100.00
Docosaheksanoic	0.14 ^b	1.12 ^a	700.00	0.13	0.19	46.15	0.07 ^b	0.30 ^a	328.57	0.08 ^a	0.00 ^b	-100.00	0.41 ^a	0.12 ^b	-70.73
SFA	7.52	7.60	1.06	6.97	7.00	0.43	8.44	8.66	2.61	7.85	7.81	-0.51	7.28	7.29	0.14
MUFA	35.62	35.47	-0.42	23.56	24.27	3.01	25.64	25.89	0.98	13.39	13.61	1.64	21.60	22.20	2.78
PUFA	56.43	55.66	-1.36	68.21	67.26	-1.39	65.69	65.79	0.15	78.01	78.15	0.18	69.21	69.87	0.95

The difference between the fresh and heated averages indicated by different letters in the same line belonging to the same genotype ($P < 0.05$) is significant

(Table 2). The percentages of saturated, monounsaturated, and polyunsaturated fatty acids varied between 7–9, 13–36, and 56–78%, respectively. To the best of our knowledge, no such investigation into the effects of heat treatment on fatty acid levels in rosehip has been published. However, in a study conducted on two soybean cultivars, the effects of heat treatment on fatty acid levels were investigated and it was determined that different heat treatments resulted in significant changes in the levels of these substances (ZILIC *et al.* 2010). In this study, we observed that the levels of some fatty acids changed significantly while those of others retained stable. The composition of fatty acids is not always stable but is influenced by a number of factors such as genetic, ecological, morphological, physiological and cultural elements (KARACA & AYTAC 2007).

CONCLUSION

Our results show that total sugar, pH and antioxidant activities of fresh and heat-treated fruits of rosehip species were not affected by heat treatment but that β -carotene was affected significantly in all species. Additionally, significant losses in total phenolics, α -tocopherol, and vitamin C were found in some species. The obtained results and the cited literature show that besides the processing procedures, growth conditions and genetic factors, environmental stresses that trigger plant defence responses such as exposure to high levels of UV light and elevated temperatures, e.g., could be important factors influencing the levels of phytochemicals. Heat treatment did not significantly affect the ratio of major fatty acids except for *R. dumalis* (MR-12 and MR-46). In conclusion, it can be concluded that temperatures which do not exceed 100°C/30 min may be used to obtain bioactive-rich rosehip extracts.

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