ORIGINAL ARTICLE

A C G publications

Rec.Nat. Prod. 10:4 (2016) 407-425

records of natural products

GC×GC-TOF/MS Chromatographic Analysis, Antioxidant Capacity and Phenolic Content of *Rosa Canina L.* at Different Maturities

Dilek Ozyurt[1,2*], Birsen Demirata[2], Resat Apak[3], Jacqueline F. Hamilton[1], Alastair C. Lewis[4] and Mustafa Z. Ozel[1*]

[1] *Department of Chemistry, University of York,York, YO10 5DD, UK*

[2] *Department of Chemistry, Faculty of Science and Letters, Istanbul Technical University, Maslak 34469, Istanbul, Türkiye*

[3]*Department of Chemistry, Faculty of Engineering, Istanbul University, 34320 Istanbul, Türkiye*

[4]*National Centre for Atmospheric Science, University of York, York, YO10 5DD, UK*

(Received April 15, 2015; Revised June 12, 2015; Accepted June 14, 2015)

Abstract: The relationship between food and health has become increasingly significant as consumers now demand healthy, tasty and natural foods which perform a useful function in the body. In Turkey, rose hip (*Rosa canina L.*) is in popular use in foodstuffs such as marmalade, pestil (a traditional Turkish sweet) and syrup or in traditional medicine, mainly as herbal tea. The composition, total antioxidant capacity (TAC) and total phenolic content (TPC) of volatiles of rose hip samples were compared in relation to their stage of maturity and collection location. Rose hip fruit and seed were analyzed using a direct thermal desorption technique coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOF/MS). The major components found in the volatile fraction released on extraction were free fatty acids. The various stages of maturity of rose hips (green, light yellow, yellow, orange and red coloured) and the place of collection (Istanbul, Mersin, York) resulted in some changes in volatile compound speciation, in TAC and TPC levels. Various extraction methods were compared; methanol extraction, traditional hot water infusion and boiling in water. Boiling in water for ten minutes was the method which demonstrated the highest extraction efficiency. TAC and TPC were determined using different electron transfer−based assays; the Cerium (IV) ions reducing antioxidant capacity, Cupric Reducing Antioxidant Capacity and Folin-Ciocalteu methods. The highest TAC and TPC were found in the red coloured (fully ripe) rose hip fruit, suggesting that colour is a suitable indicator for optimal harvesting time.

Keywords: Rose hips; maturity stage; direct thermal desorption; comprehensive gas chromatography; total antioxidant capacity; total phenolic content.

1. Introduction

Plants from the *Rosaceae* family have been used for food and medicinal purposes for a very long time. The *Rosaceae* species are known to have one of the highest vitamin C contents amongst fruit an

* Corresponding author: E-Mail: ozyurtd@itu.edu.tr; Phone:+90-212-285-72-73

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 01/01/2016 EISSN:1307-6167

vegetables and also contain carotenoids, tocopherol, bioflavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids and essential oils [1−4]. These compounds display biochemical activities such as being antioxidants, antimutagenics and anticarcinogenics [5,6]. Rose hip is the fruit of the rose plant and is typically red to orange in colour, but ranges from dark purple to black in some species. Turkey is one of the most important germplasm centres for rose species; twenty five rose species (about 25% of all rose species) have so far been reported to grow in Turkey [7]. Rose hip is known as one of the most effective remedies against hemorrhoids and diabetes mellitus in Turkish traditional medicine. The seeds and leaves of the plant have also been used against bronchitis [8]. In Turkey, there are several special rose hip products manufactured and they have been used for many purposes, such as rose hip marmalade, rose hip pestil, which is a traditional sweet and also rose hip syrup and rose hip tea made with both fruit and seeds. The fruit, leaves and seeds are boiled in water and used as a diuretic and as ingredients in common cold remedies [7,9].

Plant foods contain phenolic compounds which affect their appearance, taste, odor and oxidative stability. Although rose hips also in general contain important phenolic compounds, differences in content may exist due to the degree of maturity, genetic variation, climate, growing and storage conditions and uncertainy in total content is generated by the method of measurement used. The most advantageous harvesting time needs to be determined to help maximise the effect of rose hip when it is used for health purposes in different foods. In this study, volatile organic compounds, total antioxidant capacity (TAC) and total phenolic content (TPC) were determined during the different maturity stages of rose hip. The stage of ripeness of the rose hip samples were classified by the colour of the fruit. In the literature, colour, sugar and carotenoid content have been suggested for assessing optimal ripeness of rose hip for *Rosa dumalis, Rosa rubiginosa, Rosa spinosissima and Rosa dumalis hybrid* [10,11]. A higher content of carotenoids were achieved with a later harvesting time [11]. Uggla et al. [10] found that the colour of rose hip fruit was a good indicator of optimum harvesting time.

Several analytical methods have been developed for the estimation of TAC [12]. A basic classification of TAC assays is the underlying type of reaction, for example, electron transfer (ET) based assays or hydrogen atom transfer assays (HAT) [13]. ET based assays include trolox equivalence antioxidant capacity (ABTS/TEAC) [14,15], 2,2-diphenyl-1-picrylhydrazyl (DPPH) [16], Ferric ion reducing antioxidant power (FRAP) [17-19], Cupric reducing antioxidant capacity (CUPRAC) [20-22], Cerium (IV) ions reducing antioxidant capacity (CERAC) [23,24], and fluorometric-CERAC [25]. Generally, HAT based assays for chain-breaking antioxidants are more robust in their kinetic principles, and more sensitive, but at the same time are more time-consuming and require significant experience in chemical kinetics for application. ET based assays are more practical, flexible, and suitable for the routine testing of natural products. These commonly provide more information on the capability of natural products to reduce reactive species and scavenge stable free radicals.

The Folin−Ciocalteu method was initially used for the analysis of proteins [26]. Much later, Singleton et al. extended this assay to the analysis of total phenols in wine [27]. It has also been proposed that the Folin-Ciocalteau phenolics assay should be standardized for use in the routine quality control and measurement of antioxidant capacity of dietary supplements and other botanicals [28].

Plant volatiles normally contain a complex mixture of organic compounds. They are largely composed of a range of saturated or partly unsaturated cyclic and linear molecules of relatively low molecular mass and within this range a variety of hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetals, ethers and esters) exist. Both hydrocarbons and oxygenated compounds are responsible for the characteristic flavors and odors. Conventional one dimensional gas chromatography generally does not always provide sufficient separation for complex plant extracts even when used in conjunction with a mass spectrometer. Since plant oils contain large numbers of similar boiling point compounds, it is possible that some components can obscure lower concentration analytes of interest.

Comprehensive two-dimensional gas chromatography (GCxGC) is a fully multi-dimensional technique achieving a much increased peak capacity in a reasonable analysis time. GCxGC has been

shown to be an extremely powerful technique for the analysis of essential oils. The fast eluting peaks in GCxGC require a fast responding detector, and the addition of a high acquisition rate of time of flight-mass spectrometer provides a system with very high resolving power. The coupling of GCxGC to TOF-MS is a very effective analysis technique for complex plant extracts [29]. Sample preparation can be through offline extraction using solvents, or, through direct thermal desorption (DTD). DTD is one of a class of dynamic headspace techniques which releases organic compounds directly from the plant sample into the first GC column via a cryogenic trapping step to maintain peak sharpness. DTD with a GCxGC-TOF/MS system allows for the qualitative and quantitative analysis of volatile compounds without any sample preparation [30].

The present study was designed to evaluate the volatile compounds of rose hip samples and their TAC and TPC using a combination of CERAC, CUPRAC, Folin-Ciocalteu and GGxGC-TOF/MS methods.

2. Materials and Methods

2.1. Reagents, materials and apparatus

All chemicals used were of analytical reagent grade and double-distilled water was used throughout. Cerium (IV) sulphate tetrahydrate, neocuproine (2,9-dimethyl-1,10-phenanthroline), Folin-Ciocalteu phenol reagent, potassium sodium tartrate tetrahydrate, copper (II) chloride and copper (II) sulphate were purchased from Sigma-Aldrich (Gillingham, UK). Sodium carbonate and ammonium acetate (NH_4OAc) were supplied from Fluka Co. (Buchs, Switzerland). Sodium sulphate, sodium hydroxide, methyl alcohol, ethyl alcohol and sulphuric acid were supplied from Fisher Scientific (Loughborough, UK).Rose hip (*Rosa canina L.*) samples were collected from Istanbul Technical University campus (Istanbul, Turkey) in August and September of 2011, from the Mut Kozlar Plateau (Mersin, Turkey) in August of 2011 and The University of York campus (York, UK) in September of 2011. The maturity stage (ripeness) of the rose hip samples were classified by the colour of the fruit. Samples having a green colour were characterized as unripe then followed by the stages of light yellow, yellow, orange all the way up to the red coloured samples which were characterized as fully ripe. Samples were either fruit-without-seed or seed-only. All samples were divided into very small pieces to be dried in a desiccator at room temperature. The divided pieces were placed into the glass desiccator, which contained $CaCl_2$ at the bottom. The maximum drying time in the desiccator was 120 h [31]. Dried rose hip samples were ground enough to pass through a 1.00 mm screen. All fruit samples were stored in airtight glass jars in refrigerated conditions until they were used. All experiments were carried out in the same laboratory. The UV–vis spectra recording and absorbance measurements were made with the use of a Perkin Elmer Lambda 25 UV/VIS spectrophotometer (Cambridge, UK) equipped with a pair of matched quartz cuvettes of 1 cm light path. The GCxGC–TOF/MS system consisted of an Agilent 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph and a Pegasus III TOF-MS (LECO, St. Joseph, MI, USA). The modulator between first and second GC columns was based on a Leco (Cheshire, UK) liquid nitrogen two stage cold jet system. The first column was a non-polar BPX5 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) and the second column a BPX50 (1.5 m × 0.10 mm i.d. × 0.10 μm film thickness) both from SGE Analytical Science (VIC, Australia).

2.2. Preparation of solutions

Cerium(IV) sulphate tetrahydrate stock solution ($2.0x10^{-3}$ M) was prepared and transferred to a 100 mL flask. After the addition of 17 mL of 98% (by wt.) H_2SO_4, the flask was diluted to the line with water. Sodium sulphate solution (0.1 M) was prepared by dissolving 71 g Na_2SO_4 in 500 mL copper(II) chloride stock solution (1.0×10^{-2} M). Ammonium acetate (NH_4OAc) buffer at pH 7 and neocuproine solution (7.5×10^{-3} M) were freshly prepared in ethanol.

Folin-Ciocalteu's phenol reagent was diluted at a volume ratio of 1:3 with water prior to use. Lowry A solution was prepared from sodium carbonate such that the strength of Na_2CO_3 in 0.1 M NaOH solution was 2% (w/v). Lowry B solution was prepared from copper (II) sulphate such that the strength of $CuSO_4$ in 1% sodium potassium tartrate ($NaKC_4H_4O_6$) solution was 0.5% (w/v). Lowry C solution was prepared by mixing 50 mL Lowry A with 1 mL Lowry B at the time of measurement.

2.3.Preparation of rose hip samples for measurement of TAC and TPC

For the purpose of measuring TAC and TPC, rose hip samples first had to be prepared, that is, active organic components extracted from the plant matrix. Three methods were used to extract rose hip samples; methanol extraction, hot water infusion and water boiling.

Methanol extraction; 1 g samples of powdered rose hip were used. These were extracted in stoppered flasks using 80% (v/v) methanol. Three successive batch extractions were carried out using the stirrer. The first extraction was made with 20 mL MeOH for 60 min, the second with 20 mL MeOH for 45 min, and the third with 10 mL MeOH for 15 min. The three extracts were filtered and combined in a single graduated flask and diluted to 50 mL with 80% MeOH at room temperature.

Hot water infusion; 1 g samples of powdered rose hip were dipped separately into 250 mL of freshly boiled water in a beaker and occasionally shaken for 5 min. The rose hip solution (infusion) was allowed to cool to room temperature and filtered through a Whatman white-band filter paper to remove particulates.

Water boiling method; 1 g samples of powdered rose hip were put into 250 mL of boiling water in a beaker for 5 min, 10 min, 15 min, 20 min and 25 min. These are the times normally used when boiling rose hip and similar herbal teas. The rose hip solution was allowed to cool to room temperature, and filtered through a Whatman white-band filter paper to remove particulates and diluted to 250 mL with distillated water.

2.4.CERAC assay of total antioxidant capacity

1 mL of 2.0 x 10^{-3} M Ce(IV) solution + 7 mL of 1 M Na_2SO_4 solution + x mL of the rose hip extract were placed into a test tube and diluted to 10 mL with H_2O. The mixture was allowed to stand for 30 min at room temperature and the 320-nm absorbance (A_{320} nm) was measured. The total antioxidant capacity was determined and results expressed as g trolox equivalents dry weight [24].

2.5.CUPRAC assay of total antioxidant capacity

1 mL $CuCl_2$ solution (1.0×10^{-2} M), 1 mL neocuproine alcoholic solution (7.5×10^{-3} M) and 1 mL 1 M NH_4OAc buffer solution were added to a test tube and mixed. Following this, (x) mL rose hip extract then (1.1-x) mL H_2O were added (total volume, 4.1 mL) and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. The total antioxidant capacity was determined and results expressed as g trolox equivalents dry weight [20].

2.6.Folin-Ciocalteu assay of total phenolic content

To (x) mL of rose hip extract, (2-x) mL H_2O was added. An aliquot of 2.5 mL Lowry C solution was added, and the mixture was allowed to stand for 10 min. At the end of this period 0.25 mL Folin reagent was added, and 30 min more waiting time was allowed for stabilization of the blue colour formed. The absorbance against a reagent blank was read at 750 nm. The total phenolic content was determined and results expressed as g trolox equivalents dry weight [27].

2.7.Direct Thermal Desorption

To evaluate the volatile compounds in rose hip samples, DTD followed by analysis with GCXGC-TOF/MS was performed. There was no sample preparation, dried rose hip was directly loaded into the system.

A GCxGC-TOF/MS system was used together with a dual stage commercial thermal desorption injector. This incorporated a thermal desorption unit (TDU) connected to a programmable-temperature vaporisation (PTV) injector, CIS-4 plus (Gerstel, Mulheim an der Ruhr, Germany), using a heated transfer line. The injector was equipped with a MPS autosampler (Gerstel). Empty glass thermodesorption tubes were conditioned at 400 °C for 2 h prior to each use. Approximately 50 mg of sample of rose hip was placed into the thermodesorption tubes using tweezers to ensure no contamination of the sample. Glass wool was used to hold the sample in place. Each desorption tube was then sealed with a stainless steel / viton o-ring assembly and handled throughout the remaining analysis stages by a robotic autosampler. Initial desorption of the sample was carried out by heating the TDU from 40 °C (initial time 0.2 min) to 150 °C at a rate of 120 °C min^{-1} with a final hold time of 5 min under a helium flow of 1.5 mL min-1 in splitless mode. Volatile analytes released from this heating were cryo-focused at -40 °C in the CIS cooled with liquid nitrogen prior to injection. The CIS was then heated at a rate of 10 °C s^{-1} to a final temperature of 150 °C. Analytes were transferred splitless to the GC column during the CIS temperature ramp.

2.8.Chromatographic Analysis

The GCxGC–TOF/MS system was used. The modulator secondary oven was operated at +15 °C above the GC oven temperature. The modulation time was 5 s. The initial temperature of the first dimension column was 70°C for 1 minute and the subsequent temperature programme was a heating rate of 5°C min^{-1} until 270°C was reached and held isothermally for a further 1 minute. The initial temperature of the second dimension column was 85°C for 1 minute and a 5°C min^{-1} heating rate was used until 285°C was reached and held isothermally for further 1 min. Helium was used as a carrier gas at a constant flow of 1.0 mL min^{-1}. The columns were connected by means of a press-fit-connector. The first dimensional separation is based on separation by volatility in a non-polar column. The second dimensional separation is based on separation by polarity using a more polar column. The combination of separations produces the overall two dimensional chromatogram. Peak identification was made using TOF/MS with electron ionisation. The mass spectrometer used a push plate frequency of 5 kHz, with transient spectra averaging to give unit resolved mass spectra between 45 and 350 amu at a rate of 50 spectra s^{-1}. Mass spectra were compared against the NIST 2005 mass spectral library and Kovats indexes from the literature.

2.9.Statistical analysis

All analyses were run in triplicate and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Statgraphics Centurion XVI programme (StatPoint Technologies, Inc.,Warrenton, Virgina, USA). Significant differences between means were calculated by analysis of variance (ANOVA) procedures; $p < 0.05$ were regarded as significant.

3. Results and Discussion

3.1. GC analysis

The volatile chemical composition, TAC and TPC of rose hip samples were compared with stages of maturity and collection places. The fruit was separated from the seeds before GC analysis. The maturity stages of the fruit ranged from green to red, red meaning fully ripe. The content of main

compounds varied depending on the maturity of the plant. The results revealed very large variations in the content of free fatty acids in rose hip fruit and seed. It was found in this study that the differing maturity stages of rose hip resulted in a change in volatile compounds. Tables 1 and 2 show an inventory of the compounds identified in rose hip fruit and seed, respectively.Eighty nine compounds in rose hip fruit (without seed) were identified and they are listed in Table 1. It can be seen that the change in colour (maturity) and collection place of samples resulted in a slight change of components and relative composition of the volatile oils. In the rose hip fruit samples the main components found were acetic acid, furfural, hexanoic acid, heptanoic acid, octanoic acid, 5-(hydroxymethyl)-2-furancarboxaldehyde, nonanoic acid, 1-docosene and heptacosane. Only 16 of the compounds identified were common to all 9 samples. Rose hip fruit contains mainly free fatty acids, hydrocarbons, aldehydes, ketones and browning reaction products. Browning reaction products may negatively affect the flavour of the dried product. Rose hip contains sugar, and during drying or heat treatment, sugar produces furan and furanone types of compound [31]. Furfural, 2(5H)-furanone, acetylfuran, furaneol, and 5-(hydroxymethyl)-2-furancarboxaldehyde together with other minor ones are known to be the result of browning reactions. The chemical composition of the rose hip samples tested showed that, apart from the sample collected in York, there were no significant changes in composition. In the York sample, there were fewer compounds found and a different set of relative abundances. Table 2 lists the compounds identified in rose hip seed. Using the direct thermal desorption method with GCxGC-TOF/MS, fewer compounds (67) were seen from the seed when compared with fruit. The main compounds were mainly free fatty acids, browning reaction products, acetic acid and 1-docosene. The average percentage of free fatty acids in rose hip fruit and seed was 17.9% and 32.9%, respectively. Average percentages of browning reaction products were found to be slightly higher from the fruit (24.8%) than from the seeds (13.7%). The rose hip sample collected in York showed a notable difference in chemical composition in both its fruit and seed. Differences in the the composition of volatile compounds in samples may be due to collection time, differing chemotypes, different genotypes and geographic or climatic factors such as temperature. In this case, geographic or climatic factors could be the reason why the York, UK sample varied so much from the other two batches which were collected from Turkey. Fluctuations in the concentrations of various products with increased ripening or darkening colour of a given rose hip sample possibly arises from enzymatic or degradative conversion of initially formed compounds. In intact apple fruit, fatty acid (FA) precursors are assumed to be derived from two metabolic pathways; (i) from the catabolism of membranes or other lipid ‘storage’ pools at certain stages of ripening and maturation of fruit, causing the release of FA by the activity of lipase and possibly lipoxygenase [32] and (ii) from *de novo* FA biosynthesis followed by their oxidative degradation, accompanied by an involvement of the released FA in volatile aroma biosynthesis [33]. Therefore, the general trend involving an initially gradual increase, followed by a decrease after reaching a maximum of certain FA precursors in rose hip fruits and seeds (Tables 1 & 2) may arise from these two competitive metabolic processes of degradation and biosynthesis. As for a similar trend in fluctuations of aldehyde concentrations (Tables 1 & 2), it may be proposed that aldehydes can readily be converted to alcohols and esters in ripening fruits [34] such that when the capacity to produce esters is fully developed by suitable enzymes, aldehydes may be converted to alcohols at a rate that prevents aldehyde accumulation in excess of the concentration present in immature fruit [35].

3.2. Selection of sample preparation method for TAC and TPC measurement

It should be noted that for the purposes of selection of preparation methods and subsequent experiments, the red coloured rose hip fruit samples from York, UK were used since these were the most abundantly available to us.

The TAC and TPC of red coloured rose hip fruit (without seed) collected from York was measured and compared with those of brand X commercially produced rose hip tea. This tea was known to contain both fruit and seed. A seed-only sample was tested too. Methanol extraction, hot

water infusion and water boiling methods were employed for sample preparation as described in the part above entitled "Preparation of rose hip samples for measurement of TAC and TPC" with the purpose of investigating how the different preparation methods affected the results. Measurement of TAC was performed using modified CERAC, CUPRAC and TPC using the Folin-Ciocalteu methods. Results are shown in Table 3. Results from the Folin-Ciocalteu method are given using the unit of mmol trolox/ g sample (instead of mg gallic acid per gram) to enable direct comparison of results with other TAC methods. The results were a mean of three experiments. In the red fruit-without-seed and brand X commercial tea samples, TAC and TPC were found to be highest from water boiling extraction, then from hot water infusion, and lowest from 4:1 (v/v) methanol/water extraction. However, TAC and TPC of the seed-only samples were much lower than that of the fruit and tea and did not show any pattern. Antioxidant components in the red fruit-without-seed and tea samples seemed to be more efficiently extracted using the water boiling method and less so using 4:1 (v/v) methanol/water. This may derive from the hydrophilic - lipophilic balance of rose hip antioxidants. The TAC and TPC of the commercial rose hip tea extract were seen to be lower than the fruit sample but better than the seed sample. This tea is known to contain both fruit and seed together.

Solubility of phenolic compounds increased with higher temperatures using the boiling method. Increasing the temperature causes Maillard reaction products to be formed. Some studies have shown that Maillard reaction products may have antioxidant activities [36, 37]. In our study, we used temperatures lower than 100°C and so not many Maillard reaction products would have been formed. Increasing the boiling time above 10 minutes decreased the amounts of TAC and TPC.

3.3. Effect of boiling times using water extraction

In the literature, previous results have shown that the particle size of the sample, the ratio of the volumes of solvent to sample, the temperature of extraction and the time of extraction and heating change the quantity of volatiles extracted [30, 31]. In our study, variables of boiling time, between 5 min and 25 min, were applied to examine how this affected TAC and TPC. Figure 1 shows that a water boiling time of 10 min was found to be optimal to maximise the recovery of TAC and TPC in the rose hip fruit-without-seed sample from York. TAC and TPC increased until 10 min of boiling time was reached. The results from 15 and 20 min boiling time showed a small decrease and finally the 25 min boiling time resulted in approximately 15% degradation of TAC and TPC. As it yielded the best results in terms of amount of material extracted, we chose the water boiling method of extraction over the other methods for further studies, and boiled for the optimum time of 10 minutes. Increasing boiling time from 10 minutes also would decrease the amounts of ascorbic acid extracted which is known as a good antioxidant.

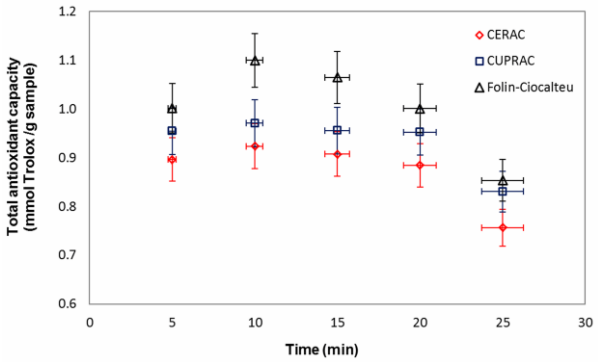

Figure 1. Optimization of water boiling extraction time of rose hip fruit (without seed) as a function of to the total antioxidant capacity and total phenolic content

Table 1. Volatile chemicals isolated from rose hip fruit-without-seed samples at different stages of maturity and from different collection locations using direct thermal desorption - GCxGC-TOF/MS.

Compound [a]	RI [b]	Istanbul					Mersin			York
		Green	Light Yellow	Yellow	Orange	Red	Yellow	Orange	Red	Red
Acetic acid	600	6.83±0.57 [c]	2.74±0.36	7.33±0.92	4.78±0.64	1.17±0.35	4.31±0.28	8.23±0.59	5.18±0.26	
Propanoic acid	668	- [d]	0.12±0.03	-	0.14±0.02	-	-	-	-	
2,3-Pentandione	700	-	-	0.55±0.12	-	0.48±0.05	0.11±0.02	1.05±0.15	0.66±0.13	
Pentanal	704	0.52±0.05	-	-	-	-	0.20±0.04	0.21±0.02	-	
Butanoic acid	763	0.68±0.09	0.56±0.08	0.48±0.05	-	0.22±0.04	0.28±0.04	0.39±0.03	0.24±0.03	
2,3-Butanediol	785	-	-	-	0.12±0.02	-	1.70±0.15	0.78±0.06	0.82±0.04	0.25±0.06
Hexanal	801	3.56±0.46	2.37±0.31	2.94±0.43	1.61±0.22	0.70±0.08	0.34±0.05	0.30±0.06	1.25±0.14	-
Methylpyrazine	819	-	0.18±0.02	-	0.24±0.03	-	-	-	-	-
Furfural	828	0.83±0.06	4.36±0.28	8.46±0.76	3.59±0.53	9.49±0.68	6.66±0.72	12.72±0.91	9.66±0.84	4.69±0.74
Acetol acetate	843	-	0.24±0.02	0.26±0.03		-	0.35±0.06	-	0.78±0.05	0.57±0.07
2-Hexenal	846	1.19±0.09	1.51±0.18	1.33±0.22	0.51±0.06	-	0.47±0.05	-	1.13±0.23	0.86±0.12
1-Hexanol	851	0.63±0.05	0.43±0.06	-	-	-	-	-	-	-
2(5H)-Furanone	871	0.39±0.05	0.85±0.09	0.30±0.06	0.27±0.07	0.63±0.08	0.80±0.04	1.33±0.15	1.25±0.34	1.35±0.14
2-Heptanone	889	-	0.26±0.08	0.14±0.05	0.13±0.03	0.11±0.02	-	0.08±0.02	0.13±0.04	-
Cyclohexanone	898	-	-	-	-	-	0.22±0.05	0.20±0.04	0.38±0.08	-
Heptanal	901	2.56±0.39	2.59±0.45	2.37±0.40	1.96±0.28	1.13±0.14	0.94±0.10	0.44±0.09	0.98±0.10	-
2,5-Dimethylpyrazine	908	0.24±0.04	0.26±0.06	-	0.27±0.06	-	-	-	-	-
Acetylfuran	910	-	0.65±0.15	-	-	1.15±0.32	1.16±0.42	1.11±0.23	1.00±0.18	0.17±0.05
Pentanoic acid	911	1.66±0.43	2.08±0.28	1.83±0.34	0.85±0.15	1.54±0.24	1.14±0.32	1.05±0.08	0.97±0.04	0.28±0.06
5-Methyl-2-(3H)-furanone	920	-	-	-	-	-	0.09±0.03	0.12±0.06	0.25±0.05	-
α-Pinene	933	0.18±0.04	0.40±0.08	0.11±0.04	1.01±0.09	0.41±0.08	-	-	-	-
Camphene	946	0.24±0.08	0.11±0.04	0.19±0.05	0.20±0.04	0.20±0.05	-	-	-	-
2-Heptenal	947	-	2.05±0.15	1.78±0.18	4.13±0.93	0.59±0.12	1.01±0.07	1.00±0.09	1.85±0.23	-
Benzaldehyde	952	0.12±0.03	0.24±0.04	0.14±0.04	0.18±0.03	-	0.19±0.05	0.16±0.06	0.31±0.05	-
Hexanoic acid	967	11.60±1.46	14.90±2.05	14.76±1.80	7.65±0.79	7.83±0.53	3.32±0.62	2.55±0.45	0.89±0.12	-

Methyl-2-furoate	969	-	-	-		-	0.69±0.05	0.91±0.05	1.18±0.08	0.65±0.07
Phenol	980	0.28±0.04	-	0.40±0.10		-	0.13±0.03	-	-	0.11±0.02
β-Pinene	981	-	-	-	0.12±0.03	0.16±0.04	-	-	-	-
6-methyl-5-hepten-2-one	985	0.69±0.08	0.67±0.09	1.99±0.29	2.53±0.56	0.63±0.14	0.36±0.12	0.58±0.06	0.96±0.11	0.93±0.12
2-Octanone	988	0.51±0.06	1.39±0.09	1.01±0.15	0.59±0.08	0.30±0.08	0.39±0.06	0.50±0.12	0.53±0.06	0.14±0.04
2-Pentyl-furan	991	0.43±0.12	0.78±0.14	0.54±0.08	0.72±0.09	0.34±0.06	0.32±0.09	0.23±0.05	0.66±0.11	-
Octanal	998	1.13±0.24	1.58±0.39	0.87±0.09	0.55±0.06	0.44±0.08	0.52±0.10	0.41±0.12	0.51±0.07	-
(E,E)-2,4-Heptadienal	1005	-	0.16±0.05	0.35±0.06	0.18±0.04	-	0.11±0.02	0.05±0.02	0.17±0.05	-
2-Ethylhexanoic acid	1027	0.20±0.05	0.19±0.07	0.30±0.08	0.24±0.06	-	0.31±0.05	0.18±0.06	-	0.09±0.03
Eucalyptol	1030	-	-	-		-	0.84±0.12	0.26±0.06	-	-
2-Ethyl-1-hexanol	1032	0.18±0.04	0.32±0.09	0.16±0.06	0.13±0.05	-	0.08±0.03	0.08±0.02	0.11±0.03	-
Benzeneacetaldehyde	1036	0.68±0.09	0.71±0.12	0.56±0.11	0.45±0.08	0.18±0.04	2.13±0.43	0.35±0.07	1.39±0.16	0.15±0.05
γ-Terpinene	1054	-	-	0.47±0.11		-	1.53±0.27	-	-	-
5-Ethyldihydro-2(3H)-furanone	1056	-	0.08±0.02	-	0.08±0.03	-	0.03±0.01	-	0.03±0.01	-
Acetophenone	1059	-	0.11±0.03	0.11±0.04	0.10±0.03	0.05±0.02	0.09±0.03	0.06±0.02	0.05±0.02	-
Furaneol	1064	-	2.95±0.59	2.70±0.88	0.33±0.12	2.44±0.40	5.22±0.81	5.81±0.89	4.86±0.64	5.18±0.95
Heptanoic acid	1083	3.33±0.43	4.31±0.26	3.81±0.88	3.11±0.63	2.72±0.45	1.21±0.26	2.43±0.66	1.51±0.57	1.14±0.23
Terpinolen	1086	-	-	-		-	1.32±0.25	-	0.72±0.15	-
2-Nonanone	1087	-	0.34±0.08	0.33±0.05	0.25±0.05	-	0.11±0.03	0.16±0.04	0.14±0.04	-
Nonanal	1100	1.87±0.44	2.45±0.39	2.05±0.29	4.32±0.75	2.40±0.33	2.52±0.29	-	1.02±0.22	-
Maltol	1106	-	-	-		0.57±0.13	0.84±0.12	0.72±0.05	0.87±0.08	0.83±0.10
Phenylethyl alcohol	1106	-	-	-		-	0.03±0.01	0.06±0.02	0.04±0.02	-
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1134	0.61±0.08	0.51±0.09	0.89±0.09	1.50±0.26	5.86±0.76	5.93±0.91	6.89±1.08	3.32±0.36	13.06±1.11
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	1139	0.31±0.09	-	0.13±0.04	0.22±0.05	-	0.21±0.06	0.19±0.04	0.23±0.05	0.56±0.12
(E)-2-Nonenal	1157	0.37±0.09	0.43±0.05	0.43±0.06	0.28±0.09	-	0.32±0.04	0.22±0/07	0.20±0.05	-
Octanoic acid	1167	5.20±0.89	6.47±0.54	4.89±0.62	4.55±0.78	3.31±0.61	3.30±0.75	3.32±0.74	2.62±0.50	-
p-Cymen-8-ol	1179	-	-	-		-	2.45±0.48	0.14±0.05	-	-
2-Decanone	1190	0.34±0.12	0.53±0.06	0.49±0.08	0.27±0.09	0.32±0.05	0.15±0.04	0.18±0.04	0.15±0.05	0.12±0.05
1,2-Benzenediol	1197	-	-	-		-	0.21±0.04	-	0.11±0.04	0.31±0.05
Decanal	1204	0.67±0.05	0.98±0.12	0.50±0.11	0.63±0.09	-	0.50±0.08	0.30±0.08	0.35±0.07	-

5-Hydroxymaltol	1205	-	-	-		-	0.30±0.05	0.25±0.04	0.17±0.06	0.57±0.09
5-(Hydroxymethyl)-2-furancarboxaldehyde	1224	-	0.60±0.07	1.11±0.14	1.15±0.15	12.08±2.06	22.38±3.49	21.56±4.12	21.82±3.90	30.05±5.52
(E)-2-Decenal	1260	0.21±0.05	0.26±0.06	-	0.16±0.04	-	-	-	0.17±0.05	-
Nonanoic acid	1267	8.46±0.95	6.27±0.83	5.33±0.67	5.08±0.95	2.78±0.43	5.92±0.81	1.04±0.16	2.88±0.67	0.48±0.09
Butyrolactone	1299	0.55±0.11	1.14±0.23	0.32±0.07	0.31±0.09	0.20±0.05	0.71±0.09	0.75±0.13	0.58±0.09	0.43±0.12
2,6,10,10-Tetramethyl-1-oxa-spiro[4.5]dec-6-ene	1313	0.17±0.05	0.23±0.08	0.27±0.06	0.07±0.02	-	-	-	-	-
(E,E)-2,4-Decadienal	1315	-	0.05±0.02	0.08±0.02		-	-	0.06±0.02	0.11±0.03	-
Decanoic acid	1364	2.39±0.48	1.65±0.23	1.69±0.47	0.89±0.14	1.56±0.44	1.18±0.31	1.61±0.16	1.19±0.25	0.83±0.12
Tetradecane	1400	0.21±0.05	0.22±0.06	0.14±0.04		-	0.20±0.05	-	0.10±0.03	-
α-Ionone	1428	-	-	-		3.06±0.51	0.15±0.03	0.31±0.07	0.72±0.06	-
6,10-Dimethyl-(E)-5,9-undecadien-2-one	1436	0.23±0.08	0.21±0.05	0.29±0.08	0.15±0.05	-	-	-	-	-
Undecanoic acid	1490	0.16±0.04	0.44±0.08	0.09±0.03	0.17±0.03	-	-	-	0.33±0.04	-
α-Farnesene	1505	-	-	0.32±0.07	0.12±0.02	-	-	-	-	-
5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-(R)-2(4H)-benzofuranone	1525	-	0.05±0.02	-		-	0.11±0.02	-	0.05±0.02	-
Dodecanoic acid	1565	1.48±0.23	0.65±0.09	0.73±0.08		1.18±0.21	0.43±0.09	0.83±0.07	-	-
Hexadecane	1600	0.74±0.08	0.31±0.07	0.30±0.05	0.18±0.05	-	0.12±0.04	0.07±0.02	0.59±0.06	-
N-Butylbenzenesulfonamide	1604	-	0.09±0.03	0.09±0.02	0.11±0.02	-	0.13±0.03	-	0.04±0.01	0.05±0.01
Dihydro-3-hydroxy-4,4-dimethyl-, (ñ)-2(3H)-furanone	1685	0.20±0.05	0.12±0.04	0.10±0.04	0.07±0.03	0.17±0.02	0.21±0.04	0.23±0.06	0.30±0.08	0.28±0.06
Heptadecane	1700	0.21±0.06	0.25±0.05	-		1.93±0.23	0.24±0.06	-	0.20±0.05	-
Tetradecanoic acid	1720	-	-	-		-	0.19±0.05	-	-	0.34±0.07
3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	1722	-	-	0.75±0.08	0.98±0.13	0.95±0.09	-	-	-	-
Octadecane	1800	-	-	-		0.39±0.05	-	0.55±0.08	-	-
2-methyloctadecane	1867	0.36±0.07	1.12±0.23	-	0.15±0.03	-	-	-	-	-
Nonadecane	1900	0.59±0.08	-	-		0.39±0.07	-	-	-	-
Dibutyl phthalate	1922	0.61±0.09	0.51±0.09	0.62±0.12	0.38±0.06	-	0.68±0.06	0.28±0.06	0.09±0.02	0.08±0.02

Methylpalmitate	1927	-	0.11±0.03	-		-	0.14±0.02	-	0.15±0.03	-
Hexadecanoic acid	1959	0.35±0.08	-	0.88±0.13		-	-	-	0.90±0.14	-
2-Methylnonadecane	1966	1.90±0.31	5.01±0.90	-	10.83±1.45	11.04±2.09	-	-	-	-
Eicosane	2000	3.67±0.56	4.39±0.64	0.93±0.13	2.17±0.24	0.26±0.11	0.95±0.12	2.72±0.54	0.70±0.10	0.27±0.05
1-Docosene	2195	9.04±1.54	2.33±0.71	6.73±0.88	13.34±2.49	10.94±1.50	2.42±0.54	5.31±0.89	5.16±0.78	1.18±0.31
Docosane	2200	3.44±0.87	2.17±0.32	4.06±0.67	1.86±0.41	0.82±0.12	-	-	-	0.24±0.05
Pentacosane	2500	0.31±0.06	-	1.07±0.09	0.96±0.14	-	-	-	-	-
Heptacosane	2700	8.87±1.18	3.20±0.54	3.30±0.87	6.54±1.23	1.94±0.43	0.50±0.12	2.24±0.47	7.23±1.38	27.88±4.84
Unknown		7.85±1.36	6.79±1.07	5.61±0.98	5.55±0.87	4.96±1.05	7.72±0.49	6.43±1.09	6.94±1.12	5.89±0.45

[a] As identified by GCxGC-TOF/MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices.
[b] Kovats retention indices (column: BPX5)
[c] Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100 (In the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte).
[d] not detected or percentage of the component is lower than 0.01%.

Table 2. Volatile chemicals isolated from rose hip seed-only samples at different stages of maturity and from different collection locations using direct thermal desorption - GCxGC-TOF/MS.

Compound [a]	**RI** [b]	**Istanbul**					**Mersin**			**York**
		Green	**Light Yellow**	**Yellow**	**Orange**	**Red**	**Yellow**	**Orange**	**Red**	**Red**
Acetic acid	600	1.80±0.23 [c]	5.05±0.33	1.79±0.09	4.44±0.89	1.65±0.34	1.26±0.32	5.94±0.90	7.84±1.02	1.22±0.32
Pentanal	704	- [d]	-	-	-	0.49±0.09	0.90±0.13	2.13±0.24	-	-
Butanoic acid	763	0.49±0.09	0.45±0.07	0.31±0.07	-	0.22±0.05	0.21±0.04	0.26±0.04	0.50±0.09	0.20±0.04
1-Octene	792	0.47±0.08	-	-	-	0.22±0.05	-	-	0.12±0.03	-
Hexanal	801	2.47±0.65	2.53±0.54	0.74±0.13	0.91±0.13	1.17±0.12	0.39±0.08	0.73±0.09	1.20±0.23	0.28±0.06
Methylpyrazine	819	-	0.21±0.05	-	0.21±0.04	-	-	-	-	-
Furfural	828	0.99±0.13	1.75±0.26	4.45±0.55	2.62±0.45	9.14±1.25	2.99±0.34	5.21±0.87	5.45±0.90	1.70±0.56
(E)-2-Hexenal	854	-	0.41±0.08	0.30±0.05	-	-	0.83±0.12	0.73±0.09	0.91±0.10	-
2-Furanmethanol	866	0.68±0.05	1.32±0.23	2.46±0.45	0.47±0.12	0.64±0.09	0.85±0.14	1.81±0.23	1.78±0.34	0.52±0.09
2(5H)-Furanone	871	0.31±0.09	0.34±0.07	0.23±0.06	0.21±0.05	0.64±0.07	0.37±0.09	1.11±0.12	0.69±0.13	0.52±0.11
2-Heptanone	889	0.21±0.04	0.20±0.05	0.14±0.03	0.21±0.05	0.17±0.03	0.09±0.03	0.11±0.02	0.17±0.03	-
Cyclohexanone	898	-	-	-	-	0.06±0.02	0.05±0.02	0.07±0.02	0.08±0.02	0.18±0.04
Heptanal	901	4.13±0.80	2.98±0.71	2.24±0.56	1.26±0.43	1.86±0.34	1.87±0.45	1.21±0.23	1.91±0.15	2.52±0.43
2,5-Dimethylpyrazine	908	0.36±0.12	0.18±0.04	-	0.37±0.04	-	-	-	-	-
1-(2-Furanyl) ethanone	910	0.17±0.03	-	0.30±0.06	0.20±0.05	0.17±0.03	0.19±0.04	0.35±0.05	0.51±0.05	-
Pentanoic acid	911	1.56±0.12	2.27±0.43	1.80±0.34	1.73±0,54	1.57±0.65	2.52±0.50	1.21±0.20	0.75±0.13	1.01±0.09
(E)-2-Heptenal	947	-	1.04±0.23	-	-	-	0.24±0.05	-	0.08±0.02	-

Benzaldehyde	952	-	0.15±0.03	0.17±0.03	0.17±0.05	0.07±0.03	0.08±0.02	0.12±0.03	0.20±0.05	0.12±0.03
5-Methylfurfural	962	0.11±0.04	0.32±0.05	0.59±0.09	0.25±0.05	0.99±0.16	0.43±0.09	1.22±0.23	1.19±0.39	0.37±0.08
Hexanoic acid	967	14.47±2.58	10.35±2.09	9.12±1.46	9.98±1.76	13.39±2.86	9.95±1.67	5.69±0.90	5.82±0.43	0.73±0.12
6-Methyl-5-hepten-2-one	985	-	0.41±0.12	0.41±0.08	0.39±0.07	-	-	0.09±0.03	0.21±0.04	-
2-Octanone	988	0.80±0.09	1.12±0.15	0.79±0.12	0.50±0.09	0.38±0.05	0.50±0.14	0.39±0.10	0.53±0.06	0.44±0.08
2-Pentylfuran	991	0.26±0.09	0.75±0.06	-	-	-	0.37±0.08	-	0.65±0.07	-
Octanal	998	1.42±0.35	1.59±0.24	4.34±0.76	0.93±0.15	1.62±0.34	0.80±0.12	0.65±0.09	0.70±0.09	1.65±0.34
2-Ethylhexanoic acid,	1027	0.39±0.09	0.27±0.05	0.23±0.05	0.23±0.08	-	0.20±0.05	0.15±0.04	0.11±0.04	0.18±0.03
2-Ethyl-1-hexanol	1032	-	0.16±0.03	0.12±0.03	0.07±0.02	-	0.06±0.02	0.05±0.02	0.08±0.02	-
Benzeneacetaldehyde	1036	0.22±0.05	0.22±0.06	0.18±0.04	0.25±0.05	-	0.22±0.04	0.14±0.03	0.35±0.06	0.08±0.02
Acetophenone	1059	-	0.08±0.02	0.06±0.02	0.08±0.02	-	0.04±0.01	0.03±0.01	0.04±0.01	0.06±0.02
Heptanoic acid	1083	4.24±0.48	4.80±0.67	4.04±0.90	4.00±0.82	2.68±0.65	4.14±0.86	2.50±0.65	1.84±0.43	2.80±0.79
2-Nonanone	1087	0.35±0.08	0.36±0.11	0.30±0.05	0.29±0.08	0.19±0.05	0.20±0.05	0.16±0.04	0.17±0.04	0.23±0.04
Nonanal	1100	2.38±0.50	3.16±0.45	3.10±0.45	2.69±0.76	2.82±0.76	1.05±0.21	2.00±0.19	2.04±0.45	1.79±0.34
Maltol	1106	-	-	-	-	-	0.07±0.02	0.21±0.05	0.41±0.08	-
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1134	-	0.21±0.05	0.78±0.12	2.47±0.66	1.11±0.15	2.12±0.34	1.75±0.34	5.82±0.90	3.24±0.54
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	1139	0.12±0.03	0.10±0.02	-	-	-	-	-	0.20±0.04	0.08±0.02
(E)-2-Nonenal	1157	-	0.43±0.09	0.32±0.06	0.30±0.05	-	0.23±0.06	0.18±0.04	0.19±0.03	0.33±0.08
Octanoic acid	1167	6.61±0.98	7.47±1.05	7.62±0.87	4.82±0.66	4.30±0.61	5.31±0.90	3.97±0.78	3.16±0.60	4.21±0.45
1-Dodecene	1187	-	0.29±0.06	-	0.26±0.06	-	-	-	-	-
2-Decanone	1190	0.56±0.12	0.54±0.09	0.38±0.08	0.49±0.09	0.48±0.05	0.29±.0.06	0.18±0.05	0.31±0.05	0.47±0.06
1,2-Benzenediol	1197	-	-	-	-	-	0.08±0.02	0.14±0.04	-	0.12±0.02
Decanal	1204	0.62±0.05	0.55±0.12	0.62±0.13	1.21±0.18	0.26±0.05	0.34±0.06	0.45±0.08	0.23±0.05	0.63±0.11
5-(Hydroxymethyl)-2-furancarboxaldehyde	1224	0.11±0.03	-	1.79±0.34	1.37±0.32	8.85±1.01	7.96±0.87	14.16±2.32	16.72±1.90	12.99±2.34
Nonanoic acid	1267	8.01±1.03	9.22±1.23	6.93±1.45	6.90±1.02	4.51±0.98	7.98±1.28	5.41±0.78	4.70±0.87	4.31±0.45
Benzoic acid	1276	-	-	-	-	-	0.09±0.03	0.08±0.02	0.13±0.02	-
Butyrolactone	1299	0.37±0.06	0.40±0.09	0.46±0.12	0.20±0.05	0.15±0.03	0.27±0.07	0.38±0.08	1.42±0.19	0.29±0.09
Decanoic acid	1364	2.68±0.40	2.04±0.48	1.33±0.34	2.15±0.45	1.18±0.43	1.48±0.32	1.04±0.23	1.02±0.25	1.34±0.37
Tetradecane	1400	0.14±0.02	0.21±0.04	-	-	-	-	-	0.17±0.04	-
Undecanoic acid	1490	1.81±0.34	1.22±0.26	0.59±0.11	-	-	0.09±0.03	0.72±0.05	0.23±0.05	0.15±0.03
Pentadecanal	1513	-	-	-	-	-	1.43±0.41	3.95±0.45	4.63±0.76	0.83±0.12
Dodecanoic acid	1565	-	1.75±0.43	-	-	0.76±0.29	0.65±0.15	0.97±0.11	0.96±0.14	1.27±0.17
Diethyl phthalate	1590	-	0.04±0.02	-	-	-	-	0.03±0.01	0.04±0.01	0.08±0.02
Hexadecane	1600	-	0.41±0.05	-	0.40±0.09	0.34±0.08	0.27±0.05	0.23±0.05	0.13±0.03	-
N-Butylbenzenesulfonamide	1604	-	0.14±0.03	0.11±0.02	0.18±0.04	0.09±0.03	0.17±0.02	0.06±0.02	0.07±0.02	0.30±0.05
Tetradecanal	1611	-	-	-	-	-	0.94±0.11	-	0.49±0.07	0.12±0.03

Dihydro-3-hydroxy-4,4-dimethyl-(ñ)-2(3H)-furanone	1685	-	0.11±0.03	0.09±0.02	0.10±0.02	-	0.05±0.01	0.06±0.02	0.17±0.03	-
Heptadecane	1700	-	0.90±0.05	0.18±0.04	-	0.26±0.05	0.66±0.11	0.87±0.10	0.48±0.09	-
Tetradecanoic acid	1720	0.36±0.07	0.91±0.13	-	-	-	0.15±0.03	0.19±0.05	1.22±0.27	0.33±0.05
2-Methyloctadecane	1867	1.23±0.25	0.69±0.10	0.76±0.12	0.20±0.05	1.08±0.	0.52±0.10	-	0.18±0.04	-
1-Nonadecene	1895	-	-	1.55±0.23	-	0.20±0.05	-	-	-	-
Dibutyl phthalate	1922	0.24±0.04	-	0.21±0.05	0.22±0.03	-	0.14±0.03	0.40±0.08	0.10±0.03	0.56±0.12
Methyl palmitate	1927	-	-	-	-	-	0.03±0.01	-	0.20±0.05	0.11±0.03
Hexadecanoic acid	1959	-	0.81±0.12	0.47±0.15	0.45±0.09	-	-	-	0.64±0.12	0.62±0.14
2-Methylnonadecane	1966	6.04±0.98	1.40±0.34	4.87±1.09	10.05±2.20	1.45±0.23	3.26±0.67	1.26±0.26	0.30±0.05	-
Eicosane	2000	3.51±0.87	2.34±0.56	1.72±53	1.69±0.49	5.74±0.87	1.51±0.43	1.45±0.42	1.24±0.33	2.61±0.28
1-Docosene	2195	11.63±2.22	12.88±2.51	10.30±1.45	16.15±3.09	8.35±1.18	10.54±2.03	9.75±1.18	3.77±0.65	6.92±1.06
Docosane	2200	7.01±1.02	1.37±0.45	3.10±0.67	3.12±0.42	2.52±0.49	5.26±0.80	0.09±0.03	-	-
Pentacosane	2500	-	1.09±0.21	-	0.46±0.05	0.14±0.04	0.55±0.10	-	-	-
Isooctyl phthalate	2530	1.44±0.28	1.76±0.60	1.91±0.46	1.69±0.24	0.73±0.17	1.97±0.71	0.89±0.12	0.67±0.13	1.82±0.30
Heptacosane	2700	4.03±0.71	2.45±0.43	9.02±1.34	5.09±0.76	11.25±1.59	7.65±0.93	8.99±1.02	7.45±1.10	32.36±5.99
Unknown		5.19±1.05	5.86±0.93	6.68±1.37	7.56±1.78	6.13±0.56	7.15±0.89	8.92±1.36	6.69±1.20	7.35±1.19

[a] As identified by GCxGC-TOF/MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices.
[b] Kovats retention indices (column: BPX5)
[c] Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100 (In the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte).
[d] not detected or percentage of the component is lower than 0.01%.

Table 3. Comparison of total antioxidant capacity and total phenolic content obtained using three sample preparation methods from red coloured rose hip fruit-without-seed (York), seed-only (York) and commercial rose hip tea samples.

Methods	Sample preparation		Fruit	Seed	Commercial tea
CERAC (mmol trolox / g sample)	Methanol Extraction		0.480±0.006	0.122±0.001	0.246±0.027
	Hot Water Infusion		0.625±0.001	0.081±0.004	0.382±0.002
	Water Boiling	5 min	0.897±0.005	0.078±0.003	0.478±0.001
		10 min	0.924±0.010	0.091±0.001	0.526±0.004
		15 min	0.908±0.010	0.091±0.005	0.554±0.003
		20 min	0.885±0.007	0.091±0.002	0.510±0.001
		25 min	0.757±0.005	0.093±0.001	0.556±0.011
CUPRAC (mmol trolox / g sample)	Methanol Extraction		0.568±0.098	0.108±0.001	0.193±0.004
	Hot Water Infusion		0.779±0.016	0.062±0.001	0.375±0.001
	Water Boiling	5 min	0.955±0.002	0.065±0.001	0.355±0.002
		10 min	0.971±0.008	0.073±0.003	0.381±0.005
		15 min	0.956±0.009	0.070±0.001	0.411±0.001
		20 min	0.953±0.002	0.065±0.002	0.490±0.012
		25 min	0.831±0.010	0.069±0.001	0.507±0.001
Folin-Ciocalteu (mmol trolox / g sample)	Methanol Extraction		0.569±0.006	0.122±0.001	0.215±0.002
	Hot Water Infusion		0.776±0.004	0.110±0.002	0.406±0.012
	Water Boiling	5 min	1.002±0.001	0.154±0.003	0462±0.002
		10 min	1.100±0.010	0.166±0.001	0.501±0.001
		15 min	1.065±0.004	0.165±0.001	0.520±0.001
		20 min	1.001±0.001	0.161±0.002	0.622±0.005
		25 min	0.854±0.008	0.164±0.001	0.746±0.003

3.4.Determination of total antioxidant capacity and total phenolic content of rose hip fruit without seed during maturation

Colour measurement of rose hip fruit has been proposed as an accurate measure of ripeness. We investigated how the stage of maturity of rose hip samples related to their TAC and TPC. Samples of green, light yellow, yellow, orange and red coloured rose hips were prepared using the water boiling method and the extracts used to compare the TAC and TPC. The results showed that the rose hip had the highest TAC and TPC in its red coloured (fully ripe) maturity stage (Table 4).The equation below (1) was used for the calculation TAC of rose hip samples according to the CERAC method [23].

$$\text{TAC (mmol-Trolox/g-sample)} = [(A_0\text{-}A_f) / \varepsilon_{TR}] \times (V_f/V_i) \times (DF) \times (V_e / m) \quad (1)$$

where A_0 and A_f are the initial and final (after sample reaction) absorbances at 320 nm respectively of the extraction solution at V_e mL volume. ε_{Trolox} is the indirect molar absorptivity of trolox, V_f and V_i are the final (diluted) and initial volumes (in mL) of the rose hip extract sample, respectively, *DF* is the dilution factor, and *m* is the g-amount of the rose hip sample initially taken for analysis. In CERAC, absorbance difference at 320 nm, i.e. $\Delta A = (A_0 - A_f)$, whereas in CUPRAC and Folin-Ciocalteu, direct absorbance at 450 nm and 750 nm, respectively, were used in TAC calculations as a result of trolox reactions with the relevant chromogens of the assays.

Dark-coloured vegetables, fruits and plants are known to be good sources of phenolics and antioxidants such as flavonoid, vitamin C, anthocyanin and carotenoids [38, 39]. Studies have shown that thermally processed vegetables, fruits and plants demonstrate different biological activities because of various chemical changes undergone during heat treatment. Some studies have demonstrated that phenolic content was reduced after heating (boiling, steaming) because of partial degradation or loss of phenolic compounds during this process [40]. On the other hand, certain antioxidant compounds such as carotenoids, tocopherol and lycopene have been shown to be relatively heat-resistant [41].

In our study, samples were collected from Istanbul (Turkey), Mersin (Turkey) and York (United Kingdom). Five different colours of rose hip samples were collected from Istanbul Technical

University Campus and the order of TAC and TPC of these were found to be; red > orange > yellow > light yellow > green. From the Mut Kozlar Plateau in Mersin, three different colours were collected and the order of TAC and TPC were found to be; red > orange > yellow. Only red coloured samples were available at the time of collection from the University of York Campus. Our results indicated that there was variation in content for different geographical collection areas for rose hips at the same stage of maturity. The highest TAC was found in the red coloured fruit from Istanbul and the highest TPC found in the red coloured Mersin samples. Conversely, the red coloured fruit from York exhibited both the lowest TAC and lowest TPC found throughout our experiments, even lower than that of the unripe green fruit from Istanbul.

For practical applications, it is important to have an accurate maturity marker to optimise TAC and TPC in the eventual plant extracts as these qualities are commercially desirable. The general harvesting period is long for rosehip. The results in this study support the suggestion that simple colour measurement be used as a judge of ripeness, although that colour in isolation is not the only factor which controls the amount of TAC and TPC that may be extracted. The highest TAC and TPC appear to be achieved by late harvesting of fruit at the red coloured stage, but it is important to note that geographical area of collection has a substantial effect on extractable material.

In the rose hip samples tested here, from three different geographic regions, although the overall complex chemical composition of volatile compounds did vary notably between samples, those volatile compounds which are well known to be responsible for TAC and TPC did not show significant differences. TAC and TPC did vary considably between samples suggesting that these variations may be due to differences in non-volatile compounds in the samples, (outwith the scope of our method of testing) such as flavonoid, vitamin C, anthocyanin and carotenoids.

In this study, the water boiling method yielded better results than hot water infusion and methanol extraction in relation to quantities of TAC and TPC extracted from the plant. Maximum TAC and TPC levels were found to be at 10 min. The fall-off after this is assumed to be due to degradation of bioactive compounds. Consumers of rose hip tea could therefore be advised to boil their rose hips for 10 min to receive maximum nutrient benefit.

The TAC and TPC were also seen to vary depending on the maturity of the rose hips. In our study, various colours of fruit, from green to red, representing the various maturity stages were investigated. TAC and TPC were lowest at the green unripe stage, and rose gradually through the various coloured stages until peaking in the red coloured (mature) fruit. It can be said, therefore, that colour is a useful marker in determining optimum harvest time of rose hip. It was obvious that TAC mainly originated from phenolics, as vitamin C content was reported to be highest at the onset of ripening when the rose-hips are a pale red colour, and lowest when the hips become over-ripe and turn dark [42].

Table 4. Total antioxidant capacity and total phenolic content of different coloured rose hip fruit-without-seed samples from different collection locations.

Sample colour	CERAC (mmol trolox/ g sample)			Folin-Ciocalteu (mmol trolox/ g sample)		
	Istanbul	**Mersin**	**York**	**Istanbul**	**Mersin**	**York**
Green	1.010±0.008	-	-	1.269±0.020	-	-
Light yellow	1.119±0.026	-	-	1.309±0.034	-	-
Yellow	1.152±0.012	0.957±0.005		1.310±0.034	1.659±0.025	
Orange	1.212±0.002	0.966±0.005	-	1.391±0.048	1.685±0.023	-
Red	1.238±0.004	1.037±0.009	0.924±0.010	1.443±0.026	1.705±0.009	1.100±0.010

The highest TAC and TPC were observed in red mature fruit. Only red coloured mature fruit was collected from York, UK although its TAC and TPC were found to be even lower than those of the most unripe green fruit from the two locations in Turkey. It was therefore deduced that climatic factors and geographical location may have a profound effect on the amounts of TAC and TPC found in rose hip.

4. Conclusion

The composition, total antioxidant capacity (TAC) and total phenolic content (TPC) of volatiles of rose hip samples were compared in relation to their stage of maturity (colour change) and collection location. This study has revealed that the commercially desirable qualities of rose hip associated with maximum TAC and TPC were found in mature red coloured fruit (rather than seed). Geographical areas of collection affected the TAC and TPC present in rose hip samples of similar maturities. This knowledge may be useful for producers who wish to predict the optimum times of harvest and select crops from most beneficial areas. The DTD-GCxGC–TOF/MS system used in this study provided a qualitative and quantitative method of analysis of volatile compounds in the samples without costly, time-consuming sample preparation steps. This may be useful commercially for producers wishing to target production of specific desirable volatile compounds from crops.

References

[1] P.A. Biacs and H. Daood H (1994). High performance liquid chromatography with photodiode-array detection of carotenoids and carotinoid esters in fruits and vegetables, *J. Plant Physiol.***143**, 520–525.

[2] X. Gao, L. Bjork, V. Trajkovski and M. Uggla (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems, *J. Sci. Food Agric.* **80**, 2021–2027.

[3] K. Szentmihalyi, P. Vinkler, B. Lakatos, V. Illes and M. Then (2002). Rose hip (*Rosa canina L.*) oil obtained from waste hip seeds by different extraction methods, *Bioresour. Technol.* **82**, 195–201.

[4] S. Nojavan, F. Khalilian, F.M. Kiaie, A. Rahimi, A. Arabanian and S. Chalavi (2008). Extraction and quantitative determination of ascorbic acid during different maturity stages of *Rosa canina L*. fruit, *J. Food Comp. Anal.* **21**, 300–305.

[5] Y. Nakamura, S. Watanabe, N. Miyake, H. Kohno and T. Osawa (2003). Dihydrochalcones: Evaluation as novel radical scavenging antioxidants, *J. Agric. Food Chem.* **51**, 3309–3312.

[6] H. Tapiero, K.D. Tew, G.N. Ba and G. Mathe (2002). Polyphenols: do they play a role in the prevention of human pathologies, *Biomed. Pharmacother.* **56**, 200–207.

[7] S. Ercisli (2007). Chemical composition of fruits in some rose (*Rosa* spp.) species, *Food Chem.* **104**, 1379–1384.

[8] O.D. Deliorman, A. Hartevioglu, E. Kupeli and E. Yesilada (2007). In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina L*. fruits, *J. Ethnopharmacol*. **112**, 394–400.

[9] S. Ercisli and M. Guleryuz (2005). Rose hip utilization in Turkey, *Acta Horticulturae*. **490**, 77–83.

[10] M. Uggla, K.E. Gustavsson, M.E. Olsson and H. Nybom (2005). Changes in colour and sugar content in rose hips (*Rosa dumalis L*. and *R. rubiginosa L.*) during ripening, *J. Hortic.Sci. Biotech.* **80**, 204−208.

[11] S.C. Andersson, K. Rumpunen, E. Johansson and M.E Olsson (2011). Carotenoid content and composition in rose hips (*Rosa spp.*) during ripening, determination of suitable maturity marker and implications for health promoting food products, *Food Chem*. **128**, 689–696.

[12] G. Cao and R.L. Prior (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum, *Clin. Chem*. **44**, 1309−1315.

[13] D. Huang, B. Ou and R.L. Prior (2005).The chemistry behind antioxidant capacity assays, *J. Agric. Food Chem.* **53**, 1841–1856.

[14] N.J. Miller, C.A. Rice-Evans, M.J. Davies, V. Copinathan and A. Milner (1993). A novel method for measuring antioxidant capacity and its application to monitoring antioxidant status in premature neonates, *Clin. Sci*. **84**, 407–412.

[15] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C.A. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* **26**, 1231–1237.

[16] A. Jimenez-Escrig, I. Jimenez-Jimenez, C. Sanchez-Moreno and F. Saura- Calixto (2000). Evaluation off ree-radical scavenging ofdietary carotenoids by the stable radical 2,2-diphenyl-1-picrylhydrazyl, *J. Sci. Food Agric.* **80**, 1686–1690.

[17] I.F.F. Benzie and J.J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP Assay, *Anal. Biochem.* **239**, 70–76.

[18] I.F.F. Benzie and Y.T. Szeto (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay, *J. Agric. Food Chem*. **47**, 633–636.

[19] R. Pulido, L. Bravo and F. Saura-Calixto (2000). Antioxidant activity of dietary polyphenols as determined by modified ferric reducing/antioxidant assay. *J. Agric. Food Chem*. **48**, 3396–3402.

[20] R. Apak, K. Guclu, M. Ozyurek and S.E. Karademir (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *J. Agric. Food Chem.* **52**, 7970–7981.

[21] R. Apak, K. Guclu, M. Ozyurek, S.E. Karademir and M. Altun (2005). Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: The CUPRAC method, *Free Radic. Res.* **39**, 949–961.

[22] M. Ozyurek, S.E. Celik, K.I. Berker, K. Guclu, I. Tor and R. Apak (2007). Sensitivity enhancement of CUPRAC and iron(III)-phenanthroline antioxidant assays by preconcentration of colored reaction products on a weakly acidic cation exchanger, *React. Funct. Polym*. **67**, 1478–1486.

[23] D. Ozyurt, B. Demirata and R. Apak (2007). Determination of total antioxidant capacity by a new spectrophotometric method based on Ce(IV) reducing capacity measurement, *Talanta* **71**, 1155–1165.

[24] D. Ozyurt, B. Demirata and R. Apak (2010). Modified cerium(IV)-based antioxidant capacity (CERAC) assay with selectivity over citric acid and simple sugars, *J. Food Compos. Anal.* **23**, 282–288.

[25] D. Ozyurt, B. Demirata and R. Apak (2011). Determination of total antioxidant capacity by a new spectrofluorometric method based on Ce(IV) reduction: Ce(III) fluorescence probe for CERAC assay, *J. Fluoresc.* **21**, 2069−2076.

[26] O. Folin and V. Ciocalteu (1927). Tyrosine and tryptophan determinations proteins, *J. Biol. Chem.* **73**, 627−650.

[27] V.L. Singleton, R. Orthofer and R.M. Lamuela-Raventos (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Meth. Enzymol*. **299**, 152–178.

[28] R.L. Prior, X. Wu and K. Schaich (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, *J. Agric. Food Chem*. **53**, 4290–4302.

[29] M.Z. Ozel, F. Gogus, J.F. Hamilton and A.C. Lewis (2004). The essential oil of *Pistacia vera L.* at various temperatures of direct thermal desorption using GCxGC-TOF/MS, *Chromatographia* **60**, 79−83.

[30] M.Z. Ozel, F. Gogus and A.C. Lewis (2006). Determination of *teucrium chamaedrys* volatiles using direct thermal desorption-comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry, *J. Chromatogr. A* **1114**, 164−169.

[31] F. Gogus, A.C. Lewis and M.Z. Ozel (2011). Analysis of black mulberry volatiles using GCxGCTOF/MS, *Inter. J. Food Prop.* **14**, 29−36.

[32] N.M.M. Paillard (1986). Evolution of the capacity of aldehyde production by crushed apple tissues, during an extended storage of fruits. *The Shelf Life of Foods and Beverages*, Elsevier Science, Amsterdam, pp. 369−378.

[33] J. Song and F. Bangerth (2003). Fatty acids as precursors for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit, *Postharv. Biol. Techno.* **30**, 13−121.

[34] I. Yamashita, K. Iino, Y. Nemoto and S. Yoshikawa (1977). Studies on flavor development in strawberries, biosythesis of volatile alcohol and esters from aldehyde during ripening, *J. Agric. Food Chem.* **25**, 1165−1168.

[35] X. Fan, L. Argenta and J.P. Mattheis (2000). Inhibition of ethylene action by 1-methylcyclopropene prolongs storage life of apricots, *Postharv. Biol. Techno.* **20**, 135−142.

[36] S. I. F. S. Martins, W. M. F. Jongen and M. A. J. S. Van Boekel (2000). A review of Maillard reaction in food and implications to kinetic modelling ,*Trend. Food Sci. Tech*. **11**, 364-373.

[37] M. Plaza, M. Amigo-Benavent, M.D. Del Castillo, E. Ibanez and M. Herrero (2010). Facts about the formation of new antioxidants in natural samples after subcritical water extraction, *Food Res. Internat*. **43**, 2341-2348.

[38] E. Cieslik, A. Greda and W. Adamus (2006). Contents of polyphenols in fruit and vegetables, *Food Chem*. **94**, 135–142.

[39] A. Sass-Kiss, J. Kiss, P. Milotay, M.M. Kerek and M. Toth-Markus (2005). Differences in anthocyanin and carotenoid content of fruits and vegetables, *Food Res. Internat*. **38**, 1023–1029.

[40] N. Turkmen, F. Sari and Y.S. Velioglu (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables, *Food Chem*. **93**, 713–718.

[41] V. Dewanto, X. Wu, K.K. Adom, and R.H. Liu (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, *J. Agric. Food Chem.* **50**, 3010−3014.
[42] P. Bakos, G. Verzar Petri, and G. Lukacs (1981). Grading of rose-hips in terms of colorimetric data, *Hung. Sci. Instrum*. **52**, 15−18.