Grigoriadou K *et al*. (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 4, Article number 14200
DOI:10.15835/nbha52414200
Research Article

# Unveiling the nutritional value: Phytochemical profiling of Greek *Rosa canina* L. germplasm across ripening stages and fertilization treatment

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## Abstract

*Rosa canina* L. is among the woody species which thrive through diverse habitats and is distinguished for its high nutritional value. In recent years *R. canina* L. has raised awareness due to its high demand in cosmetology and pharmacology. This study focuses on the results of a three-year experimental site including four *R. canina* L. genotypes treated with two fertilization regimes (conventional, organic) and harvested under four ripening stages. The results indicate the most suitable period for harvesting the rosehip fruit in terms of ascorbic acid, antioxidant capacity and total phenolic compounds. This study recommends the first two ripening stages in order to achieve the highest concentration of ascorbic acid (4.53 mg $g^{-1}$ F.W.). The last ripening stage came across as being the most appropriate stage for the highest total phenolic content (31.2 mg GAE $g^{-1}$ FW) and in the meantime, this study highlights that the distinct ripening stages do not fluctuate the levels of total antioxidant capacity. Overall, the current study tries to identify the nutritional potential of domesticated *R. canina* L. and specify which of the ripening stages and fertilization regimes maximize its post-harvest value.

***Keywords:*** ascorbic acid; fertilization regimes; ripening stages; total antioxidant capacity; total phenolic content

## Introduction

*Rosa canina* L. usually referred as dogrose represents a false fruit which inheres to the Rosaceae family (Chrubasik *et al*., 2008). Nowadays, a lot of scientific work is stemming from the need to discover alternative

*Received: 14 Oct 2024. Received in revised form: 16 Dec 2024. Accepted: 16 Dec 2024. Published online: 18 Dec 2024.*
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

methods of feeding the population in order to address malnutrition and so far, the nutritional value of *R. canina* L., in terms of medicinal applications of rosehip extracts, including its anti-inflammatory, antioxidant and antimicrobial properties have been highlighted (Yilmaz and Ercisli, 2011; Peña *et al*., 2023; Kunc *et al*., 2023; Serrano *et al*., 2023). In addition, the rich composition of rosehips in vitamin D and E renders them ideal for boosting the immune system and maintaining health (Waghmare and Bharati, 2023). At the same time, it can prevent cardio-vascular diseases by reducing blood pressure and help improve obesity-related problems (Andersson *et al*., 2012). The content of ascorbic acid (Kunc *et al*., 2023), total phenolic compounds (Serrano *et al*., 2023), antioxidant capacity (Kunc *et al*., 2023), color parameters (Medveckienė *et al*., 2023) and mineral elements (Kazaz *et al*., 2008) of rosehip fruit has already been studied and concluded that rosehip fruits possess high content of phytochemicals which contribute to the protection of the human immune system. Especially, the presence of high levels of ascorbic acid plays a pivotal role in human health as it limits the level of free radicals (Nojavan *et al*., 2008).

However, in many cases, the aforementioned phytochemical characteristics are inextricably linked to the genotype of each plant species (Grigoriadou *et al*., 2023) along with the environmental conditions prevailing. This may stem from the fact that each genotype under specific environmental frame, can accumulate divergent levels of phytochemicals due to unforeseen stress factors. Consequently, the phytochemical content may fluctuate according to genotype and environmental type of effects. Therefore, many researchers make efforts to highlight the nutritional value of rosehip fruits in different regions of the world with priority to the natural habitat of each genotype which they consider to be, in most cases, the main prerequisite for their high nutritional profile (Ercisli, 2007; Roman *et al*., 2013; Bozhuyuk *et al*., 2021; Kunc *et al*., 2023).

The biochemical composition of rosehip fruits also depends on the germplasm, the growing place and the stage of ripening (Kovacs *et al.,* 1999; Kovacs *et al*., 2004). Rosehip fruits ripen over a 3-5-month interval, from summer to autumn, during which they change their color gradually from green, to yellow, to orange, to light red and finally to dark red (Stoian-Dod *et al*., 2023). This transition affects the accumulation of phytochemicals in the fruits as they ripen. A significant number of scientific studies have attempted to determine the phytochemical content of rosehip fruit during the biological ripening process, resulting in significant differences not only in phytochemical (Grigoriadou *et al*., 2023; Barros *et al*., 2011) but also in agronomic level (Medveckienė *et al*., 2023). Thus, determining the optimal harvest time is crucial for enhancing the quality and nutritional content of the final product (Barros *et al*., 2011; Elmastaş *et al*., 2017; Dolek *et al*., 2018; Al-Yafeai *et al*., 2018; Medveckienė *et al*., 2021; Medveckienė *et al*., 2023).

The aim of the present study was to investigate the phytochemical content of four discreet *R. canina* L. genotypes, classified into four ripening stages according to their color. For this purpose, color, total phenolic content, antioxidant capacity, ascorbic acid content and mineral elements content of the rosehip fruit samples were determined to assess the most suitable ripening stage for each of the measured parameters.

## Materials and Methods

*Site cultivation and Greek Rosa canina germplasm*

Rosehip fruits were collected from the experimental field of Greek *Rosa canina* L. germplasm cultured in the pilot field in Institute of Plant Breeding and Genetic Resources (IPBGR) of Hellenic Agricultural Organization DIMITRA in Thermi Thessaloniki Greece (ELGO-DIMITRA). The experimental design included the cultivation of 4 genotypes Greek *R. canina* L. germplasm (GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674) under 3 fertilization treatments: Conventional (C), Organic (O) and no treatment Control (Co) within an experimental field layout which is described in detail in previous work (Grigoriadou *et al*., 2023). For this study, rosehip fruits were obtained from 3-year-old plants

in a single harvest and then classified into 4 successive stages per genotype and fertilization treatment according to their color in order to assess the optimal stage of the rosehip fruit in terms of phytochemical characteristics. The fruits, after being classified into 4 distinct stages which we hereafter mention as "1st Ripening Stage"' (1st RS), "2nd Ripening Stage" (2nd RS), "3rd Ripening Stage" (3rd RS) and "4th Ripening Stage" (4th RS) (Figure 1), were subjected to the phytochemical analyses presented below.


**Figure 1**. Greek *R. canina* L. rosehip fruit. (**1st RS**): Indicates the first ripening stage (yellow). (**2nd RS**): Indicates the second ripening stage (orange). (**3rd RS**): Indicates the third ripening stage (light red). (**4th RS**): Indicates the fourth ripening stage (dark red).

*Determination of rose hip fruit color*

Rosehip fruit color was determined using a Minolta colorimeter (CR-400 Minolta, Osaka, Japan), which was initially calibrated with a standard factory white plate. Measurements were obtained by placing the head of the chromometer at a point on the rosehip fruit. The values were recorded in a coordinate system L*, a*, b*, in which the parameter L* represents the brightness (values from 0 for black to 100 for white), the value a* represents the color gradient from green (negative a*) to red (positive a*) and the value b* represents the color gradient from blue (negative b*) to yellow (positive b*).

*Quantification of total phenols content*

The quantification of total phenols was carried out in extracts of fruit flesh using the Folin-Ciocalteu method (Scalbert *et al*., 1989; Asami *et al*., 2003), which is based on the production of a colored (cyan) product during the oxidation of polyphenols by a mixture of phosphotungstic and phosphomolybdic acid.

Specifically, one (1) g of frozen plant issue was placed in plastic tubes and then 10 mL of extraction buffer (70% acetone, 0.5% acetic acid) was added. The mixture was vortexed and placed at 4 °C overnight in dark. Afterwards, 200 μL of extract was placed in a glass test tube and then 1 mL of Folin - Ciocalteu (Folin-Ciocalteu: $H_2O$ in a ratio of 1:10) and 800 μL of 7.5% $Na_2CO_3$ solution were added. The mixture was stirred and placed in a water bath at 50 °C for 5 minutes. The mixture was then allowed to come to room temperature and the optical density at 760 nm was measured on a microplate spectrophotometer (Tecan infinite M200 PRO). For quantification, a reference curve was constructed using gallic acid as a standard (0-100 μg) and the results were expressed in equivalent mg gallic acid/$g^{-1}$ fresh tissue weight.

*Quantification of antioxidant capacity*

The antioxidant capacity of the fruits was determined by the DPPH free radical method and with the same extraction method as in the determination of total phenolic content [one (1) g of plant issue in 10 mL of extraction buffer (70% acetone, 0.5% acetic acid)]. Afterwards, 100 μL of extract was placed in glass bottles and mixed with 2.9 mL of DPPH solution (100 μM DPPH dissolved in methanol). The solutions were then dark-adapted for 2 h at room temperature. The optical density of the mixture was then measured at 517 nm on a microplate spectrophotometer (Tecan infinite M200 PRO). A reference curve was constructed to quantify the antioxidant capacity of the extracts using Trolox as a standard. The results were expressed in equivalent mg Trolox/$g^{-1}$ fresh tissue weight.

*Quantification of ascorbic acid concentration*

For the quantification of ascorbic acid, 3 ml of metaphosphoric acid (3%) were added to 1 g of powdered fruit tissue. The mixture was rapidly stirred and then placed in the cold (4 °C) for 5 minutes. The procedure was repeated twice. The mixture was then centrifuged for 15 minutes, 11000 × g 4 °C, and the supernatant was collected into a 2 ml tube, centrifuged again for 10 minutes, 15000 × g 4 °C, collected into a new 2 ml tube and centrifuged for last time, 10 minutes 16000 × g 4 °C. The final supernatant was filtered through a 0.45μm porosity PTFE filter. Ascorbic acid was measured in High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS). Each sample was eluted through an Infinity Lab Poroshell 120 EC-C18 column (3.0 ×150 mm, 2.7 μm). The column temperature was 20 ◦C and a flow rate 0.6 mL $min^{-1}$. The mobile phase consisted of (solvent A) aqueous formic acid (0.1%, v/v) and (solvent B) acetonitrile and the injection volume was 5 μL. The gradient elusion was performed as follows: 0–5 min, 100–0% B; 6–15 min, 85–15% B; 15–17 min, 20–80% B; 17–25 min, 100–0% B. DAD acquisition ranged from 210 to 254 nm. Nitrogen (N2) was used as drying gas at a flow rate of 12 L $min^{-1}$, the drying gas temperature was 350 ◦C, nebulizer pressure at 45 psig and capillary voltage was 3500V. Selective ion monitoring mode (SIM) was used for mass acquisitions. For data acquisition and processing, Agilent OpenLab LC-MS software was used.

*Quantitative determination of inorganic nutrients*

The macronutrient and trace element content of fruits was determined according to Bremner (1960). Ground tissue (1 g) of dried fruits or leaves was weighed in a capsule and placed in an incinerator for 5 hours at 550 °C. At the end of the procedure, 5 mL HCl (6N) were added to the ash. Distilled water ($dH_2O$) was added to a final volume of 50 mL and thwn the mixture was filtered. The macroelements (P, K, Ca, Mg, Na %) and trace element (Mn, Zn, Fe, Cu ppm) content of the tissue was determined by inductively coupled plasma optical emission spectrometry (ICP–OES) system (PerkinElmer, Avio 220 Max). Nitrogen content was measured according to the Kjeldahl method using the Vapodest 50s system (Gerhardt, Konigswinter, Germany). Ground tissue of each sample (0.25 g) was placed in digestion flasks, to which a catalyst tablet and 10 mL of sulphuric acid (98 %) were added. At first, flasks were heated at 100 °C for 20 minutes and then at 425 °C for 2 h. Then, 5 mL of doubly distilled water ($ddH_2O$) and 5 mL of sodium caustate (10 N) were added. The distillate was collected in 50 mL of a 2% boric acid solution ($H_3BO_3$) followed by titration with HCl (0.1 N) until the pH stabilized at 4.5.

*Statistical analysis*

Statistical analysis of the experimental results was performed by analysis of variance (ANOVA) using the SPSS statistical package (SPSS v22.0., Chicago, USA). Statistically significant differences were determined using Duncan's multiple range test for a significance level of $\alpha$= 0.05. Data were split in each ripening stage to access the main effects of genotype and fertilization regime. All values correspond to the mean (± standard error of the mean). Graphs were drawn using Microsoft Excel.

## Results

*Rosehip fruit color*

In terms of rosehip fruit color in the 4 genotypes, it is reflected that the 4 ripening stages represent 4 different successive color stages of the rosehip fruit as the color index L* represents the brightness of the fruit color which gradually decreases as it reaches the 4th stage of maturity resulting in a rosehip fruit with the lowest L* (Figure 2). Similarly, the indexes a* and b* gradually increase and decrease, respectively, as the rosehip fruit color transitions from yellow to orange, light red and dark red, respectively (Figure 2). The fertilization regime did not seem to differentiate fruit color (Figure 2).

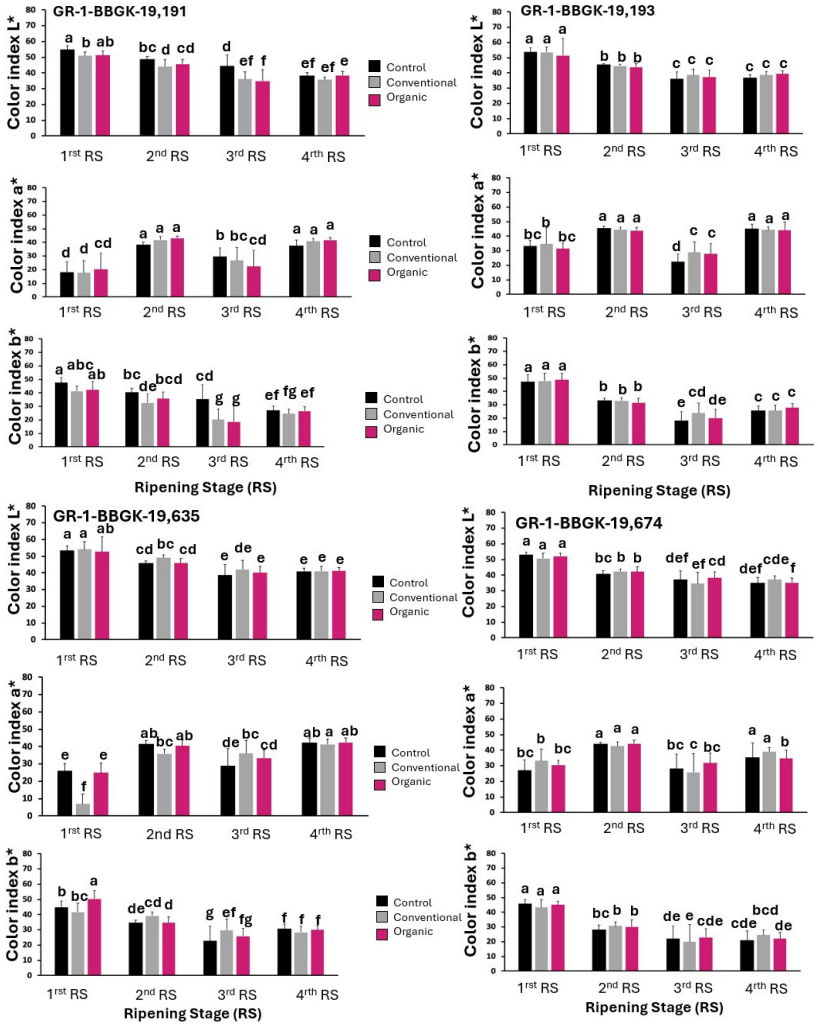


**Figure 2.** Color characteristics of fruits of the genotypes *Rosa canina* L. GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 after treatment with conventional and organic fertilization compared to the control at 4 distinct ripening stages

Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p<0.05$.

*Determination of total phenolic content*

Regarding the ripening stages, the fruits of the 4th RS had the highest content of total phenols. Specifically, in GR-1-BBGK-19,191 germplasm, the fruits of the 4th ripening stage had a higher content of total

phenols compared to the fruits of the three previous stages, while at this stage they did not differ according to the fertilization treatment that was applied (Figure 3, $p<0.05$). Also, in GR-1-BBGK-19,193 germplasm, the fruits of the 4$^{th}$ RS showed the highest content of total phenols compared to the fruits of the three previous stages, but the fruits of the control and the conventional fertilization treatment presented differences compared to those of the organic treatment (Figure 2, $p<0.05$). In GR-1-BBGK-19,635 germplasm, rosehip fruits of the organic fertilization regime of the 4$^{th}$ RS resulted to the highest total phenolic content compared to 1$^{st}$ 2$^{nd}$ and 3$^{rd}$ RS and fertilization regimes (Figure 2, $p<0.05$). As far as GR-1-BBGK-19,674 germplasm is concerned, the fruits of the 4$^{th}$ RS control treatment regime had the highest total phenolic content (31.20 mg GAE g$^{-1}$ FW) compared to the organic and conventional fertilization regimes (25.39 mg GAE g$^{-1}$ FW, 25.61 mg GAE g$^{-1}$ FW, respectfully) of the same ripening stage and showed significant differences to all the previous ripening stages and the fertilization regimes (Figure 4, $p<0.05$).

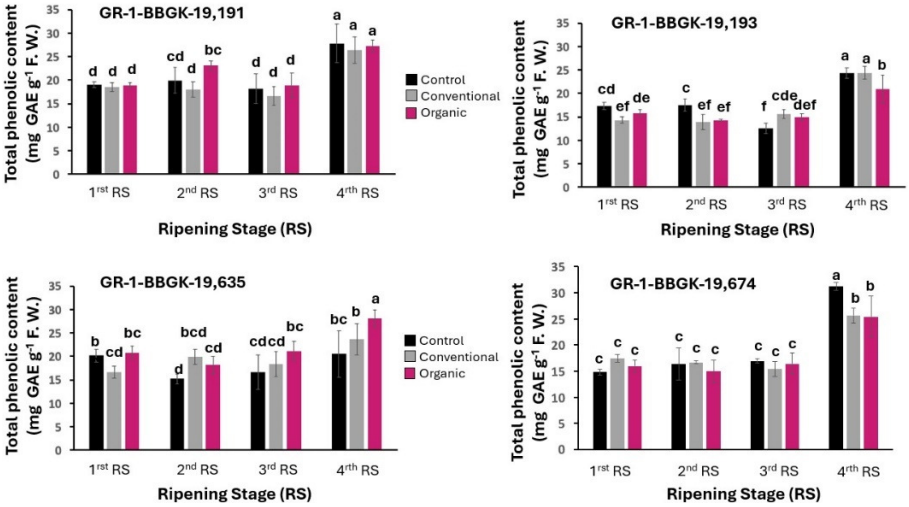


**Figure 3.** Concentration of total phenolics in rosehip fruits of *Rosa canina* L. genotypes from GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 after treatment with conventional and organic fertilization compared to the control and at 4 stages of ripening

Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p<0.05$.

Comparing the total phenolic content within the 4$^{th}$ RS it is observed that in genotype GR-1-BBGK-19,193 the organic fertilization regime brought on lower value than the organic fertilization regimes of genotypes GR-1-BBGK-19,635 and GR-1-BBGK-19,191. Alongside, conventional fertilization regimes did not seem to affect the phenolic level between genotypes at 4$^{th}$ RS. In summary, the comparison between genotypes at the 4$^{th}$ RS gave slightly better results for GR-1-BBGK-19,191, GR-1-BBGK-19,674, GR-1-BBGK-19,635 germplasm (Figure 4)

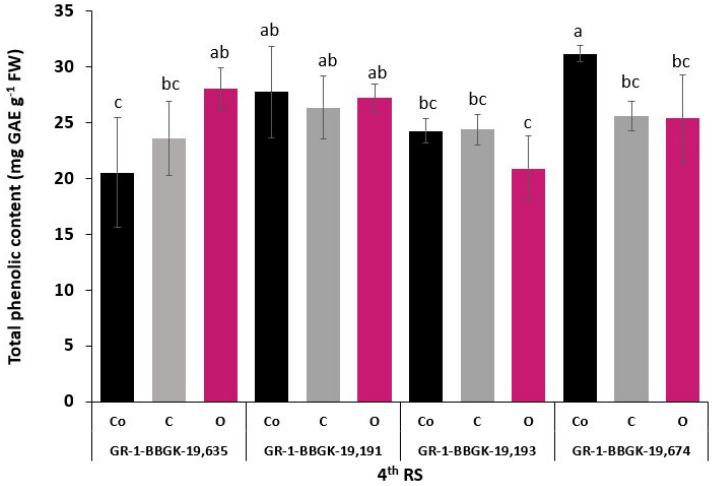


**Figure 4.** Concentration of total phenolics in rosehip fruits of *Rosa canina* L. genotypes from GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 after treatment with conventional (C) and organic (O) fertilization compared to the control (Co) at 4th ripening stage
Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p<0.05$.

*Antioxidant capacity*

In GR-1-BBGK-19,674 germplasm, no significant effect of fertilization on the antioxidant capacity of the fruits was observed. Regarding GR-1-BBGK-19,191 germplasm, in the first 3 RSs, the antioxidant capacity was significantly lower in the control treatment (Figure 5, $p<0.05$). In the 1st RS of GR-1-BBGK-19,191 germplasm, conventional fertilization regime resulted in higher value in antioxidants (23.18 mg Trolox $g^{-1}$ tissue) compared to organic treatment (13.73 mg Trolox $g^{-1}$ tissue) and control (7.58 mg Trolox $g^{-1}$ tissue) (Figure 5, $p<0.05$). On the contrary, no significant difference was found between the fertilization regimes of the 4th RS of the same genotype (Figure 5).

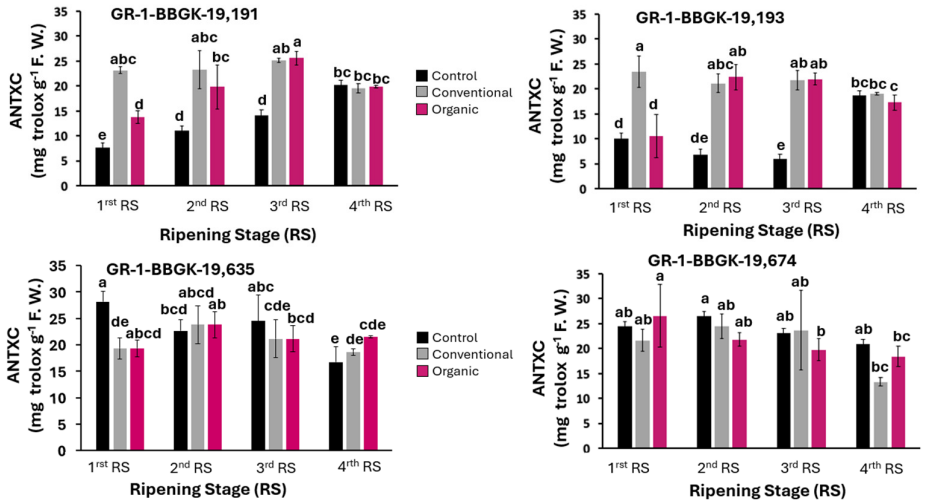


**Figure 5.** Antioxidant capacity (ANTXC) in rosehip fruits of *Rosa canina* L. genotypes from GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 after treatment with conventional and organic fertilization compared to the control at 4 stages of ripening
Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p<0.05$.

*Ascorbic acid*

In GR-1-BBGK-19,191 genotype, a significant decrease in ascorbic acid concentration was observed from the 3rd stage of ripening onwards, irrespective of fertilization regime. Especially, at the first 2 stages of ripening, conventional fertilization caused a significant increase in ascorbic acid in GR-1-BBGK-19,191 germplasm, while organic fertilization caused a decrease in ascorbic acid at the 2nd stage of ripening compared to control and conventional fertilization (Figure 6, $p<0.05$). In GR-1-BBGK-19,193 germplasm, the concentration of ascorbic acid was similarly increased in rosehip fruits of the first 2 ripening stages compared to those of the 3rd and 4th stages while in the 1st stage of ripening, conventional fertilization caused an increase in ascorbic acid compared to organic treatment and no treatment (control) (Figure 6, $p<0.05$). Additionally, in the 3rd RS, organic fertilization caused an increase, while conventional fertilization caused a decrease in ascorbic acid content compared to the control. In GR-1-BBGK-19,635 germplasm, the reverse case was found in comparison to GR-1-BBGK-19,193, i.e. conventional fertilization caused a decrease in concentration in the 1st stage but an increase in the 3rd stage compared to the other two treatments. Moreover, in GR-1-BBGK-19,635 germplasm, the levels of ascorbic acid decreased significantly in the last 2 stages of maturity in both control and organic fertilization. Likewise, in GR-1-BBGK-19,674 germplasm, in the first 2 stages of ripening, organic fertilization caused an increase in ascorbic acid levels compared to control and conventional fertilization which also resulted in an increase but only in the 2nd RS compared to control (Figure 6, $p<0.05$).

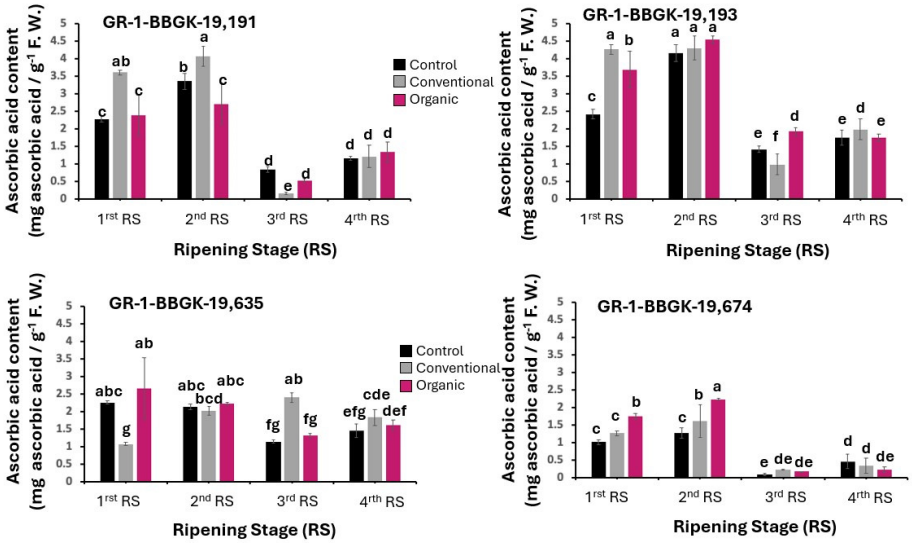


**Figure 6.** Ascorbic acid content in rosehip fruits of *Rosa canina* L. genotypes from GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 under treatments of conventional and organic fertilization compared to the control at 4 ripening stages

Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p<0.05$.

It was evidenced that the highest ascorbic acid content came from the first two stages of maturity (Figure 6) and therefore a comparison within the first 2 stages of maturity was assessed between the studied genotypes and fertilization regimes (Figure 6). The lowest value of ascorbic acid concentrations was found in the conventional fertilization of GR-1-BBGK-19,635 of the 1st RS (1.06 mg AsA $g^{-1}$ FW) and in the GR-1-BBGK-19,674 germplasm of the 1st and 2nd RS (1.26-1.74 mg ascorbic acid $g^{-1}$ FW) without showing any significant differences between them (Figure 6, $p=0.05$). As for the highest ascorbic acid values, these were determined in the organic fertilization regime of the 2nd RS of GR-1-BBGK-19,193 (4.53 mg ascorbic acid $g^{-1}$ FW) and in

the conventional and in organic fertilization regime of 2nd RS of GR-1-BBGK-19,193 without presenting any significant differences among them (Figure 7, $p$=0.05).

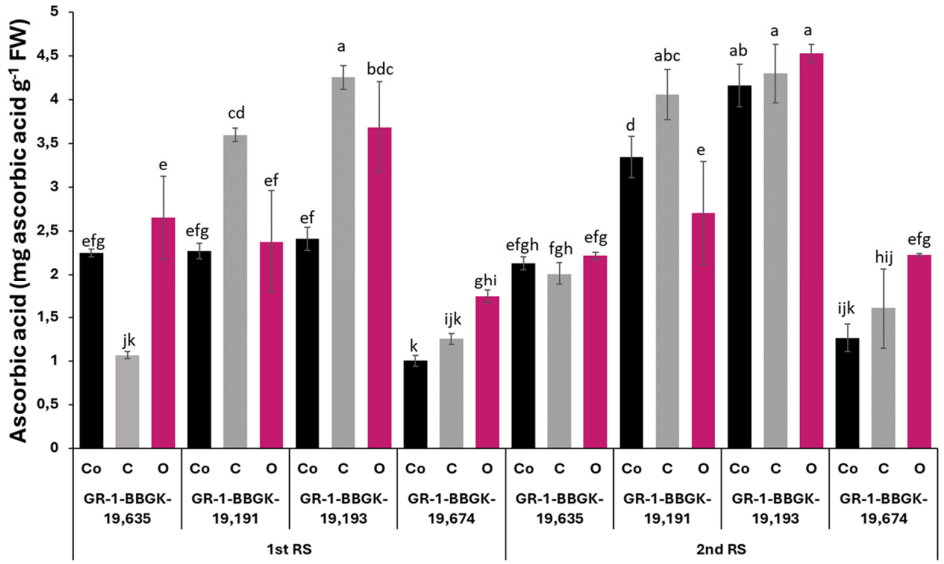


**Figure 7.** Ascorbic acid content in rosehip fruits of *Rosa canina* L. genotypes from GR-1-BBGK-19,191, GR-1-BBGK-19,193, GR-1-BBGK-19,635 and GR-1-BBGK-19,674 under treatments of conventional (C) and organic (O) fertilization compared to the control (Co) at 1st and 2nd ripening stages (RS)
Different letters indicate statistically significant differences based on Duncan's multiple range post hoc test for significance level $p$<0.05.

*Mineral nutrient content of rosehip fruit (4th RS)*

Regarding the effects of fertilization regimes on the macro-elements, in GR-1-BBGK-19,191 genotype did not show any differences in element content (Table 1). In GR-1-BBGK-19,674, after the application of C fertilization N and K contents were increased compared to the control (Table 1). Meanwhile, Ca and Mg showed a decreasing rate in both C and O treatment compared to control (Table 1). In GR-1-BBGK-19,635 C fertilization, Mg showed a higher value (0.15% $g^{-1}$ DW) compared to the O and Co (0.12% $g^{-1}$ DW and 0.13% $g^{-1}$ DW respectively) (Table 1, $p$<0.05). Additionally, in GR-1-BBGK-19,193 N content increased in C fertilization compared to Co (0.97% $g^{-1}$ DW and 0.77% $g^{-1}$ DW respectively) (Table 1, $p$<0.05).

**Table 1**. Concentrations of macro-elements measured in rosehip fruits of germplasm of GR-1-BBGK-19,191, GR-1-BBGK-19,674, GR-1-BBGK-19,635, GR-1-BBGK-19,193 genotype (G), under conventional fertilization (C), organic fertilization (O), and control (Co) at 4th ripening stage expressed as % content $g^{-1}$ DW for macro-elements

| G | FR | N | P | K | Ca | Mg |
|---|---|---|---|---|---|---|
| GR-1-BBGK-19,191 | Co | 0.73± 0.02 a | 0.11± 0 a | 0.74± 0.07 a | 0.51± 0.12 a | 0.13± 0.01 a |
| | C | 0.92± 0.07 a | 0.12± 0 a | 0.89± 0.11 a | 0.49± 0.03 a | 0.12± 0 a |
| | O | 0.88± 0.07 a | 0.12± 0 a | 0.84± 0.14 a | 0.51± 0.04 a | 0.12± 0 a |
| **G** | **FR** | **N** | **P** | **K** | **Ca** | **Mg** |
| GR-1-BBGK-19,674 | Co | 0.79± 0.04 b | 0.13± 0 ab | 1.01± 0.05 b | 0.46± 0.05 a | 0.15± 0.01 a |
| | C | 1.04± 0.02 a | 0.14± 0.02 a | 1.16± 0.14 a | 0.35± 0.05 b | 0.13± 0 b |
| | O | 1.01± 0.07 ab | 0.13±0.01 ab | 1.07± 0.19 a | 0.35± 0.01 b | 0.12± 0 b |
| **G** | **FR** | **N** | **P** | **K** | **Ca** | **Mg** |
| GR-1- | Co | 1.07± 0.04 a | 0.11± 0 a | 0.87± 0.05 a | 0.43± 0.04 a | 0.13± 0 b |

| | | | | | | |
|---|---|---|---|---|---|---|
| | C | 1.09± 0.1 a | 0.1± 0 a | 0.83± 0.01 a | 0.44± 0 a | 0.15± 0 a |
| | O | 0.85± 0.08 a | 0.1± 0 a | 0.8± 0.1 a | 0.4± 0.04 a | 0.13± 0 b |
| **G** | **FR** | **N** | **P** | **K** | **Ca** | **Mg** |
| GR-1-BBGK-19,193 | Co | 0.77± 0.06 b | 0.12± 0 a | 0.91± 0.04 a | 0.63± 0.09 a | 0.17± 0.01 a |
| | C | 0.97± 0.01 a | 0.12± 0 a | 0.95± 0.09 a | 0.59± 0.06 a | 0.17± 0 a |
| | O | 0.89± 0.06 ab | 0.11± 0 a | 0.88± 0.04 a | 0.58± 0.05 a | 0.17± 0.01 a |

Different letters within each column, separately between each genotype, indicate significant differences (Duncan's multiple range test, p<0.05). Means (± SD, n = 3) that do not share the same letter within each element column are significantly different (Duncan multiple range test, $p < 0.05$).

As for the micro-elements measured, Mn content decreased in O treated rosehips compared to Co and C treatments in GR-1-BBGK-19,191 and GR-1-BBGK-19,635 genotypes (Table 2), while in GR-1-BBGK-19,193 genotype C fertilization increased Mn content compared to Co and O treatments (Table 2). Moreover, in GR-1-BBGK-19,191 genotype C and O fertilization decrease Zn content, while in GR-1-BBGK-19,635 genotype the exact different pattern was reported with the content of Zn to be increased in C and O fertilization (Table 2). Finally, in GR-1-BBGK-19,191 germplasm, Cu content showed an increase in the C and O fertilization regimes compared to control, while in 1-BBGK-19,635 C fertilization resulted in the highest content on Cu compared to the other two treatments (Table 2).

**Table 2** Concentrations of micro-elements measured in rosehip fruits of germplasm of GR-1-BBGK-19,191, GR-1-BBGK-19,674, GR-1-BBGK-19,635, GR-1-BBGK-19,193 genotypes (G), under conventional fertilization (C), organic fertilization (O), and control (Co) at 4$^{th}$ ripening stage expressed as ppm for micro-elements

| **G** | **F** | **B** | **Mn** | **Zn** | **Fe** | **Cu** |
|---|---|---|---|---|---|---|
| GR-1-BBGK-19,191 | Co | 14.54±0.94 ab | 22.96± 4.7 b | 7.63± 0.78 a | 9.01± 0.52 b | 3.15± 0.78 b |
| | C | 15.8± 1.42 a | 29.18± 3.76 a | 9.59± 0.77 a | 11.68± 1.17 a | 4.17± 0.26 a |
| | O | 13.13± 1.2 b | 24.62± 1.66 b | 9.59± 0.57 a | 11.84± 1.6 a | 4.16± 0.42 a |
| **G** | **F** | **B** | **Mn** | **Zn** | **Fe** | **Cu** |
| GR-1-BBGK-19,674 | Co | 16.28± 0.81 a | 27.85± 2.75 a | 9.07± 2.3 a | 12.46± 1.8 ab | 3.91± 0.23 b |
| | C | 16.12± 0.92 a | 29.98± 0.82 a | 8.56± 0.26 b | 13.94± 2.75 a | 4.94± 0.23 a |
| | O | 14.39± 1.01 a | 19.81± 1.42 b | 8.41± 1.94 b | 11.28± 0.65 b | 4.56± 0.3 a |
| **G** | **F** | **B** | **Mn** | **Zn** | **Fe** | **Cu** |
| GR-1-BBGK-19,635 | Co | 13.5± 0.32 a | 22.36± 3.8 a | 6.74± 0.77 b | 9.63± 1.39 a | 4.46± 0.13 b |
| | C | 13.07± 0.22 ab | 25.28± 2.93 a | 7.62± 0.61 a | 11.98± 2.41 a | 5.03± 0.23 a |
| | O | 11.66± 1.33 b | 16.68± 0.88 b | 7.59± 0.31 a | 11.87± 4.55 a | 3.99± 0.38 c |
| **G** | **F** | **B** | **Mn** | **Zn** | **Fe** | **Cu** |
| GR-1-BBGK-19,193 | Co | 15± 2.46 a | 24.55± 1.63 b | 8.47± 1.22 a | 9.18± 0.73 a | 4.23± 0.45 a |
| | C | 15.77± 1.5 a | 33.92± 7.7 a | 8.97± 1.23 a | 9.15± 0.28 a | 5.09± 0.46 a |
| | O | 13.4± 1.17 a | 21.72± 0.9 b | 6.89± 0.4 a | 10.73± 0.79 a | 4.34± 0.22 a |

Different letters within each column, separately between each genotype, indicate significant differences (Duncan's multiple range test, p<0.05). Means (± SD, n = 3) that do not share the same letter within each element column are significantly different (Duncan multiple range test, $p < 0.05$).

## Discussion

*Rosa canina* L. is among the species with high scientific interest as many studies have been conducted for the evaluation of its high nutritional value and its numerous medical applications including the establishment of novel subproducts or nutritious foods, or substances with protective or curative usage in specific illnesses and pathologies (Chrubasik *et al*., 2008; Peña *et al*., 2023; Kunc *et al*., 2023; Serrano *et al*., 2023). It is well

known that genotype affects the plant interactions with the environment and its way to accumulate the nutrients so as to finally express its unique profile (Ercisli, 2007; Kunc *et al*., Roman *et al*., 2013; Bozhuyuk *et al*., 2021). At the same time, flowering and ripening period seem to have an excessive effect on rosehip quality (Kovacs *et al*., 2004). Herein, for the first time, rosehip fruits from 4 ripening stages treated with 3 different fertilization regimes and their response were evaluated.

*Color parameters, phenolic content, antioxidant and ascorbic acid concentration of Greek Rosa canina Germplasm as affected by four distinct ripening stages*

In the present work the four levels of ripeness were determined based on the distinctness in color. Dolek *et al*. (2018) used the same methodology for the first harvest stages but modified the last two based on fruit softening. The L-index showed a downward trend as the fruit ripened and gradually darkened. At the same time, the a* index gradually increased, while the b* index gradually decreased in all measured genotypes and consequently, at the 4$^{th}$ stage of harvesting the rosehip fruit was noticeably darker and redder than the fruit at the first stage of ripening (Figure 2). Medveckiene *et al*. (2023) have also monitored the alterations in rosehip fruit during different ripening stages on different *Rosa* species but without considering the parameter of fertilization (Medveckiene *et al.,* 2023). These color modifications align with the biosynthesis of pigments like carotenoids and anthocyanins during ripening (Kapoor *et al*., 2022).

The phenolic content across all measured genotypes, fertilization regimes and ripening stages averaged a total of 19.05 mg GAE g $^{-1}$ in the present study surpassing the results of (Macit *et al*., 2023) which resulted in an average of 10.74 mg GAE g $^{-1}$. In contrast, our findings were inferior to the results in two studies measuring total phenolic content (Al-Yafeai *et al*., 2018), in which they resulted in 78-102 mg GAE g $^{-1}$. This variance is likely attributable to differences noted in the soil and climate conditions along with the fertilization regimes. The effect of the different cultivation years should also be mentioned, as a factor, on the concentration of phenolics, since as mentioned by Kunc *et al*. (2023), in most categories of phenolic compounds the interaction between genotype and year was found to be statistically significant.

Notably, at the 4$^{th}$ ripening stage phenolic content was higher than the 1$^{st}$ stage of ripening following the same trend with L-index (Figures 2, 3). The dark red color of rosehip fruits could be explained by the high amount of polyphenols as it is well known that fruits with red color are rich in phenolic compounds and antioxidants (Hidalgo and Almajano, 2017). Dolek *et al*. (2018) came to the same conclusion as their results showed the highest total phenolic content when rosehips had already developed a fully dark red color. In the present study only in genotype GR-1-BBGK-19,635 organic fertilization treatment resulted in higher phenolic acid content compared to the other treatments (Figure 3). Regarding antioxidant capacity, it was not affected by the ripening stages and fertilization regimes (Figure 5). However, in a research effort on *Rosa rugosa* L. (Al-Yafeai *et al*., 2018), while in the early two stages there were no differences (9 and 9.1 mmol Trolox equivalent/100 g respectively), in the last stage of maturity there was an increase (15 mmol Trolox equivalent/100 g) in the concentration of antioxidants ($p$<0.05). Correspondingly, Dolek *et al*. (2018) noted that the antioxidant activity increased as the fruits ripened.

As for ascorbic acid (AsA) content, many researchers investigated the vitamin C content in rosehip fruit between different cultivars and ripening stages (Medveckienė *et al*., 2021) and concluded that *Rosa canina* L. had lower vitamin C content in the last ripening stage compared to the first two ripening stages. These results are in accordance with our findings which indicate that, in three out of four studied genotypes GR-1-BBGK-19,191, GR-1-BBGK-19,193 and GR-1-BBGK-19,674, there was a significant decrease (Figure 6, $p$<0.05) in ascorbic acid between 1$^{st}$ and 4$^{th}$ RS. A reduction in vitamin C content through the ripening stages of rosehip fruit was also highlighted in *Rosa rugose* species (Al-Yafeai *et al*., 2018). It is worth to be noted that in the 1$^{st}$ RS, genotypes GR-1-BBGK-19,191 and GR-1-BBGK-19,193 that treated with conventional fertilization showed higher values in AsA content compared to control and organic regime, a trend that genotype GR-1-

BBGK-19,191 kept at the 2nd RS as well (Figure 7). Regarding the AsA content in *R. canina* L., Kovács and Tóth (1999), measured 200-483 mg/100 $g^{-1}$ between 1996 and 1997 highlighting the increased AsA content rosehip possess as well as the fluctuation of phytochemical content between different growing periods (Kovacs *et al*., 2004).

In general, the analysis of phytochemicals in the studied genotypes coupled with RS, showed that genotypes GR-1-BBGK-19,191, GR-1-BBGK-19,674, GR-1-BBGK-19,635, were superior in total phenolic content, and at the same time genotype GR-1-BBGK-19,193 reached the higher value in terms of ascorbic acid content, especially in the first 2 stages. Therefore, to find the optimal range of total phenolic content, antioxidant capacity and ascorbic acid it is necessary to study different genotypes under distinct fertilization regimes and different maturity stages. Our results show that for the highest content of ascorbic acid the harvest should be done at the first 2 stages of ripening, for the highest content of phenolic compounds at the 4th stage of ripeness, while for antioxidants substances, ripening stage does not seem to affect the stage of ripeness (Figure 1).

*Mineral nutrient content analysis of Greek Rosa canina at 4th RS*

Minerals have a versatile role in fruit, as on one hand they characterize their nutritional value and on the other hand they relate with physiochemical characteristics and quality (Dias *et al*., 2023). Rosehips are rich in minerals which content differs between ripening stages and species/cultivars (Medveckienė *et al*., 2021; Medveckienė *et al*., 2022). In our research, differences in mineral content four genotypes a fact a fact that indicate intraspecific variation (Table 1). As for instance, in genotype GR-1-BBGK-19,674 N and K showed an increase in conventional treated rosehips while Ca and Mg decreased in conventional and organic treated rosehips (Table 1). As for micro-elements, uch as Mn, Zn, and Cu also exhibited genotype-wise responses to fertilization. Specifically, in genotype GR-1-BBGK-19,191 Mn decreased after conventional treatment, Zn also decreased after conventional and organic fertilization regime, but Cu increased after those two treatments (Table 2). In the species of *Rosa*, previous studies have revealed differences in macro- and micro-element accumulation fact that supports our findings regarding intraspecific diversity (Ercisli, 2007).

*The effect of fertilization regime in phytochemical characteristics of Rosa canina genotypes*

In terms of fertilization regimes, previous studies in various plant species highlighted differences in accumulation of sugars, vitamin C and total flavonoids in organic treated fruit (Hallmann, 2012). The organic growing system had a positive effect in tomato quality parameters including phenolic compound content and nutritional value (Hallmann, 2012). In raspberry, conventional system displayed higher yield, the fruit quality was not altered between organic and conventional system, while the levels of phytochemicals were higher with the organic farming (Moreno *et al*., 2019). In the Rosa genotypes investigated in the current study, it is a very interesting finding that regarding minerals conventional fertilization rosehips had similar results to organic treated fruit, but different from the control ones, depending on the genotype (Tables 1, 2). Ivanišová *et al*. (2023) concludes that *R. canina* L. extracts contain a significant quantity of mineral contents, without the external application of fertilization, resulting in 2.67 mg $kg^{-1}$ Cu, 13.20 mg/$kg^{-1}$ Zn, 13.20 mg $kg^{-1}$ Mn and 7.30 mg/$kg^{-1}$ Fe. Given that the values shown in the present study are superior, we can highlight the importance of the effect of fertilization regimes in rosehip mineral content (Table 2). Meanwhile, phenolic acid content was higher in organic system of fertilization in genotype GR-1-BBGK-19,635 and AsA had higher values after conventional fertilization genotypes GR-1-BBGK-19,191 and GR-1-BBGK-19,193 (Figures 4, 6). Those findings could be a foundation for future research in *Rosa canina* L. genotypes and their phytochemical profile under different fertilization regimes not only in physiochemical but also in molecular level. Also, they provide a useful tool for designing future cultivation programs to choose the genotype with the highest interaction with a specific fertilization regime that will result in the receipt of a higher number of active substances and therefore of fruit with higher nutritional value.

## Conclusions

The cultivation of rosehip is coming to the fore, mainly due to its high content of antioxidant compounds. To further investigate the nutritional value of this species, the interaction of the rosehip genotype along with different fertilization regimes and distinct harvesting stages should be examined thoroughly. This study suggests that for the extraction of the highest amount of ascorbic acid the most suitable ripening stages are the 1$^{st}$ and 2$^{nd}$. Regarding the total antioxidant capacity, maturity stage is independent, while for total phenolic content the most suitable harvest time coincides with the 4$^{th}$ maturity stage. These findings provide a foundation for future research exploring molecular-level responses and the improvement of cultivation programs to maximize nutritional value. Nevertheless, to further investigate these results, more genotypes combined with different cultivation techniques should be tested.

## Authors' Contributions

Conceptualization EM, KG, KP, KK; Data curation KK; Formal analysis KG, GT, KK; Funding acquisition EM, KG, KP; Investigation KK, KP, KG, GT; Methodology KG, KP, EM, KK; Supervision KG, EM; Validation KG, KK; Visualization KG, KK; Writing - original draft KK, KG, GT; Writing - review and editing KG, GT.

All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

## Acknowledgements

This work was supported by the RESEARCH—CREATE—INNOVATE (project code: T2EDK-02333, Green_Wild_ ROSE.gr), entitled "Development of a value chain for the Greek "wild rose" (*Rosa canina* L.) applying good agricultural practices for the production of cosmetic raw materials via innovative green extraction processes.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article

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