Vitamin C content and antioxidant activity of red currant (*Ribes rubrum* L.) juices

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Red currants (*Ribes rubrum* L.) belong to Grossulariaceae family and the *Ribes* genus. They are sweet and sour, nutrient rich berries that have shown antioxidant, antibacterial, antiseptic, cardioprotective, and anti-inflammatory effects. The aim of this study was to determine the vitamin C content and antioxidant potential of fruit juices from six varieties of red currants - Redpoll, Makosta, Stanza, Jonkheer van Tets, Rolan, and Rondom. Fresh, undamaged fruits were pressed into juice and stored at -18 °C. Ascorbic and dehydroascorbic acid were determined by high-pressure liquid chromatography. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) system as well as β-carotene bleaching assay. The results showed that the juice of the Redpoll variety had the highest vitamin C content (66.52±2.9 mg/100 g juice), while the juice of the Stanza variety had the lowest content (6.23±0.28 mg/100 g). The Redpoll variety juice also showed the strongest antioxidant activity (IC_{50} = 1.76±0.25 mg/mL), while the juice of the Rolan variety showed the weakest antioxidant activity (IC_{50} = 6.65±0.84 mg/mL). Thanks to its favorable cultivation properties and numerous potential and proven positive health effects, red currant is becoming the subject of increasing research. The results of this study can serve as an incentive for the use of red currants and their products in nutrition, as well as for the selection of nutritionally high-quality varieties.

Keywords: red currants; vitamin C; antioxidative activity; berries; juice

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1. INTRODUCTION

Currants (*Ribes* sp.) are berries popular for their sour-sweet taste and high nutrient content. Currants belong to the family Grossulariaceae, genus *Ribes*, which counts around 150 species. They are originally from the northern hemisphere and are grown worldwide as fruit and for their decorative features. The most famous types of currants are white (*Ribes album* L.), red (*Ribes rubrum* L.) and black (*Ribes nigrum* L.).
Red currants are a rich source of vitamins, minerals, sugar, as well as flavonoids and other polyphenolic compounds (USDA, 2024; Zdunić et al., 2016). Thanks to the presence of polyphenolic compounds and partly vitamin C, red currants show antioxidant activity (Benvenuti et al., 2004). In addition, antibacterial, antiseptic, cardioprotective and anti-inflammatory activity of red currant fruit has been established (Berk and Tuna, 2017; Cvetković et al., 2023). One study proved the antioxidant, anti-inflammatory and antiplatelet activity of *R. rubrum* fruit extract in mice with diabetes (Gülmez et al., 2022). The results of this study indicate that red currant extract may be useful in preventing diabetes complications.
Red currant is widely used in the food industry to obtain various products such as juices, jams, dessert toppings, ice cream, and liqueurs. Also, it is increasingly used in the cosmetic industry to obtain care products and perfumes (Milivojevic et al., 2012). Due to the high content of bioactive compounds, there is a possibility of its use in medicine and pharmacy.
In addition to fresh fruits, red currant fruit juices are a significant source of bioactive compounds. Consequently, there is an increasing need to examine the chemical composition and biological effects of these products. Red currants represent a valuable source of primary metabolites, sugars and organic acids, which play a major role in defining the distinctive flavor of the fruit. Additionally, they are rich in secondary metabo-

lites, particularly polyphenols, where three primary classes can be identified: flavonoids, tannins, and phenolic acids. Among the flavonoids, anthocyanins—specifically cyanidin- and delphinidin-3-O-glucoside—stand out as dominant compounds, along with key flavonols like quercetin and kaempferol, and notable flavanols such as catechin and epicatechin (Benvenuti et al., 2004; Djordjević et al., 2010; Milivojevic et al., 2012; Podskalská et al., 2024). The most abundant vitamin in red currant is vitamin C, the amount of which in fresh fruits ranges from 45.80 mg/100 g to 67.50 mg/100 g (Djordjević et al., 2010). Environmental conditions, cultivation systems, and genotypes have the greatest influence on the content of vitamin C in red currant fruits. The amount of vitamin C in fruits can also change depending on the ripening stage. The intensity and quantity of sunlight during the vegetative period can also affect the amount of vitamin C in the fruits because vitamin C is synthesized from sugars that are produced in the process of photosynthesis. Fruits exposed to sunlight contain higher amounts of vitamin C than those in the shade. Vitamin C primarily exists in its reduced form, known as ascorbic acid (ASC), with a smaller amount (about 5-10%) present in its oxidized form - dehydroascorbic acid (DASC). The oxidation of vitamin C occurs readily in aqueous solutions, leading to an increase in the proportion of dehydroascorbic acid. Factors such as the presence of oxygen, enzymes, heavy metal ions (particularly Cu^{2+}, Ag^{+}, and Fe^{3+}), along with high temperatures and alkaline pH, can promote this oxidation. The oxidized form of vitamin C can be easily converted back to ASC both in the human body and in foods through the action of various reducing agents such as 1,4-dithiothreitol (DTT). However, DASC is utterly unstable and it can also undergo irreversible degradation, resulting in 2,3-diketogulonic acid, which does not possess vitamin activity (Russell, 2004).

This work aims to determine the content of vitamin C, before and after reduction, as well as the antioxidant potential of the juices of six varieties of red currant - Redpoll, Makosta, Stanza, Jonkheer van Tets, Rolan and Rondom. The results will offer valuable insight into which variety(s) has the highest vitamin C content and antioxidant activity providing possible guidance for future selection practice.

2. MATERIALS AND METHODS

2.1. Preparation of materials

Six red currant varieties juices were analyzed in this work. Currants were grown with minimal use of chemicals (integrative protection system) in Radmilovac, Serbia and harvested in the period of full maturity. Juices were made from fresh, undamaged fruits by manual squeezing, and in this way, the membranes and seeds were separated from the pulp. Juices were stored in sealed vials at -18 °C for further testing.

2.2. Reagents and standards

The ascorbic acid standard and butylated hydroxytoluene (BHT) was purchased from Supelco, Buchs, Switzerland. α-linoleic acid, 1,1 diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), and 1,4-dithiothreitol (DTT) were obtained from Sigma Chemicals Co (St Louis, Mo., U.S.A.); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2 carboxylic acid (Trolox) and β-carotene from Fluka, Buchs, Switzerland; chloroform from Carlo Erba Reagents; meta-phosphoric acid p.a from Honeywell Reidel-de Haën. Acetonitrile (HPLC grade) used for analysis was J.T.Baker. The water used was purified with TKA Smart 2 pure deionization system (Thermoscience, Niederelbert, Germany).

2.3. Determination of vitamin C content in red currant juices

Ascorbic acid was quantified by a high-pressure liquid chromatography (HPLC) (Brubacher et al., 1985; Ohta and Harada, 1996). The amount of 2.5 g of juice was dissolved in 25 mL of 4.5% metaphosphoric acid. The sample was additionally dissolved in an ultrasonic bath for 10 minutes and centrifuged for 15 minutes at 10,000 rpm. The supernatant obtained in this way was used to determine ASC. The amount of DASC was determined when 1 mL of a reducing agent (50 μM 1,4 dithiothreitol - DTT) was added to 1 mL of the previously prepared sample. After a 10-minute reaction at room temperature, this solution was filtered through a membrane filter (0.45 μm) and 20 μL was injected into the HPLC. The amount of ascorbic acid quantified after reduction represents the total amount of ascorbic acid (TASC) in the sample. Dehydroascorbic acid was calculated as the difference between content of total ascorbic acid and ascorbic acid (DASC=TASC-ASC). All the results are expressed in mg of ascorbic acid per 100 g of juice.

Analysis of vitamin C content in juice was performed on an Agilent 1100 (Agilent Technologies, Palo Alto, Calif, U.S.A.) equipped with a diode array (DAD) detector, automatic sampler and control system. The separation of the components was performed on a Merck Purospher STAR RP-18e analytical column (150×4.6 mm i.d., 5 μm particle size). Phosphate buffer (40 mM) and methanol in a ratio of 92:8 under isocratic conditions were used as the mobile phase. The flow rate was 0.8 mL/min at room temperature and values were detected at an absorbance of 244 nm. Standard solutions of ascorbic acid (1, 10, 50, 100, 150 and 200 μg/mL) were injected in triplicate for each concentration to obtain a calibration curve.

2.4. Determination of antioxidant activity in the 2,2-diphenyl-1-picrylhydrazyl system

The method involves the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals in the presence of the examined antioxidant, whereby a non-radical compound is formed and the absorbance decreases due to the discoloration of the radicals. Determining the antioxidant activity of red currant juice in the DPPH system involves testing the ability of the juice to "catch" free radicals, and is performed according to the method described by Cuendet et al (1997). One mL of the DPPH ethanolic solution (0.05 mM) was added to the juices of the tested varieties of red currant (4 mL) with vigorous shaking. The resulting solutions stand for 30 minutes in a dark place at room temperature. The absorbance of the samples was measured on a UV-VIS spectrophotometer (Evolution 60 Thermo Scientific, Madison, USA) at 550 nm, as well as the control consisting of a mixture of solvent and DPPH solution, using the solvent as a blank. Inhibition of free radicals was calculated according to the formula:

$$\% \text{ inhibition of DPPH} = (A_C - A_S/A_C) \times 100$$

In the equation, A_C represents the absorbance of the control reagent, and A_S represents the absorbance of the sample. Lines were constructed showing the dependence of increasing juice concentrations and their inhibition percentages. Using the resulting equations, the results are expressed as IC_{50} (sample concentration that neutralizes 50% of free radicals). The antioxidant activity was compared with the well-known antioxidant ascorbic acid.

2.5. β-carotene bleaching assay

This method determines the antioxidant capacity of the sample by measuring their ability to prevent oxidative loss of β-carotene in the β-carotene/linoleic acid emulsion. The method was developed based on the spectrophotometric measurements of Koleva et al (2002). The emulsion was prepared in

the following way: 2 mg of crystalline β-carotene was dissolved in 10 mL chloroform. Linoleic acid (25 μL) and 180 mg of Tween® 20 are added to one mL of this solution. After complete evaporation of chloroform with a vacuum evaporator at 40 °C, 50 mL of oxygenated water was added and the emulsion thus obtained is shaken until it becomes clear. Aliquots of the emulsion (0.16 mL) were pipetted into the wells of the microtiter plates in which previously added dilution series of juices (0.04 mL). The microtiter plates were then stirred on a mixer for microtiter plates. After mixing, the initial absorbance (A0) was read on an ELISA reader (Multiskan Ascent Thermo Labsystems Elisa No 354, Thermo Fischer scientific) at a wavelength of 450 nm, the plates were incubated for 2 hours at 55 °C, after which absorbance (A_2h) was read again. The antioxidative activity was calculated according to the following formula:

$$\% \text{ inhibition} = (A_2h/A0) \times 100$$

BHA, BHT, Trolox, and ascorbic acid were used as positive controls. The results were expressed as the concentration of the sample that inhibits the loss of 50% of β-carotene (IC_{50}) and it was calculated from the concentration/% inhibition curve.

2.6. Statistical analysis

All results of antioxidant activity and vitamin C content of red currant juices were presented as three individual measurements ± standard deviation. The data were analyzed with the ANOVA method and the Tukey post-hoc test ($p<0.05$) to show statistically significant difference between the samples. Statistical data processing was performed using the SPSS20.0 program.

3. RESULTS

3.1. Vitamin C content in red currant juices

The vitamin C content of the tested juices is shown in Table 1. Based on the obtained results, the juice of the Redpoll variety had the highest amount of vitamin C before and after reduction (33.14 ± 2.21 and 66.52 ± 2.90 mg/100 g of juice, respectively). The chromatograms of vitamin C content in the Redpoll juices were presented in Figure 1a and Figure 1b, respectively.

Table 1. Vitamin C content (mg/100 g) in red currant juices before and after reduction (TASC) and calculated quantity of dehydroascorbic acid (DASC)

Red currant juice samples	Before reduction	After reduction (TASC)	DASC (DASC=TASC-ASC)
Redpoll	33.14±2.20	66.52±2.90a	33.38
Rolan	5.41±0.13	10.96±0.92b	5.55
Jonkheer van Tets	11.62±0.66	23.52±1.45c	11.90
Stanza	3.21±0.10	6.23±0.28b	3.02
Rondom	15.68±1.09	31.68±1.56c	16.00
Makosta	17.46±0.86	35.46±1.44c	18.00

[a,b,c]Different letters in columns indicate a statistically significant difference between samples (Tukey test, $p<0.05$). Results are presented as the mean value of three individual measurements ± standard deviation (except DASC).

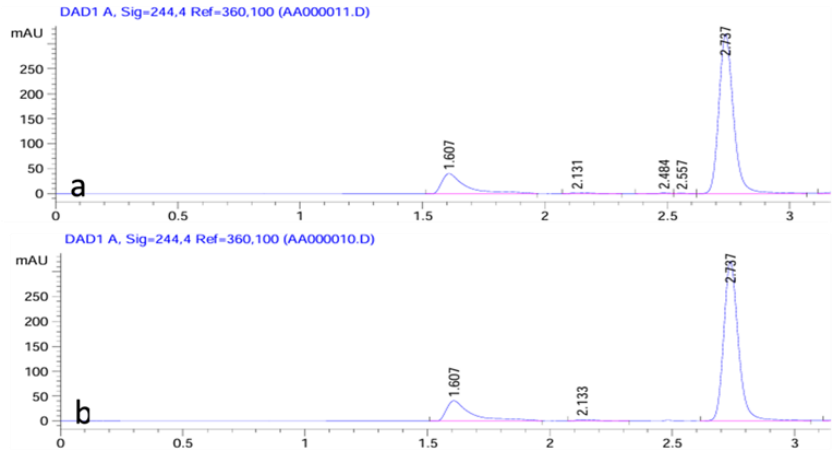

Fig. 1. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Redpoll juice

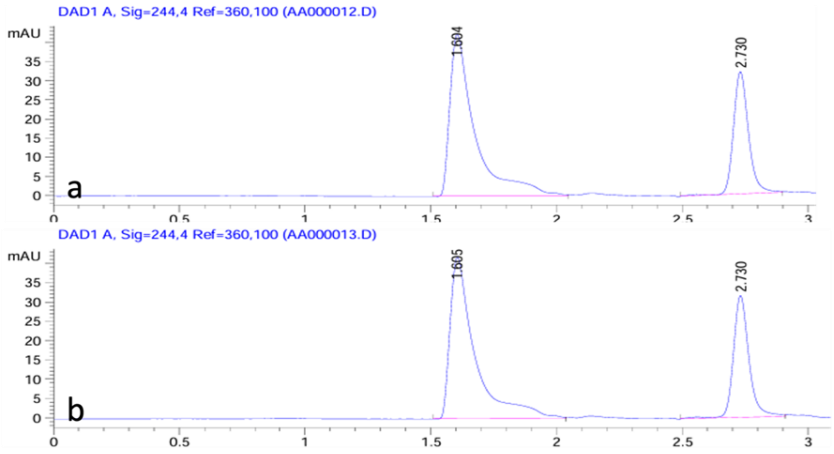

Fig 2. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Stanza juice

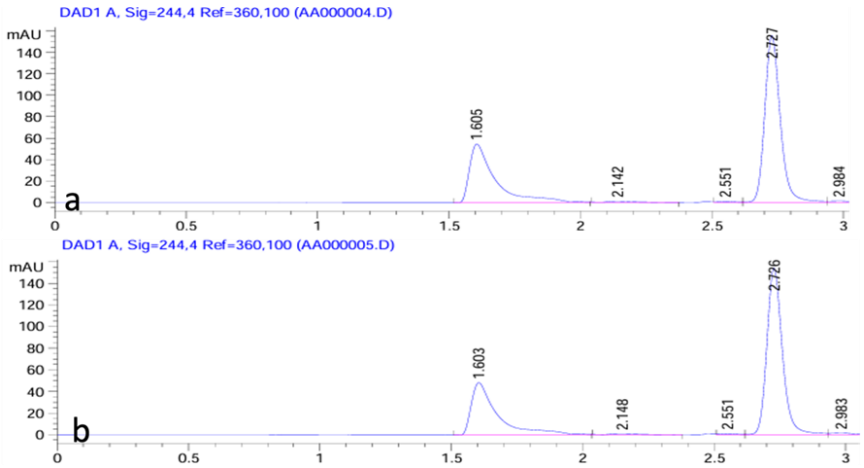

Fig3. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Rondom juice

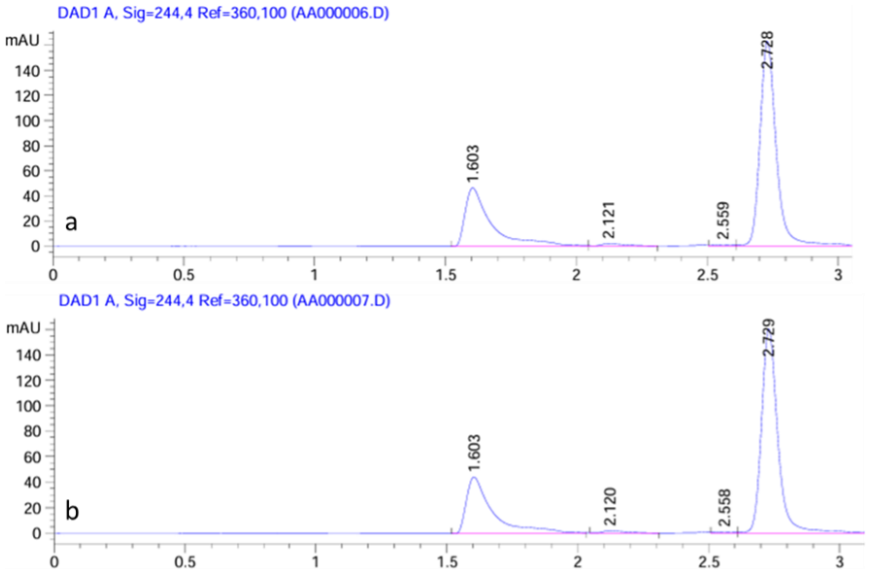

Fig 4. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Makosta juice

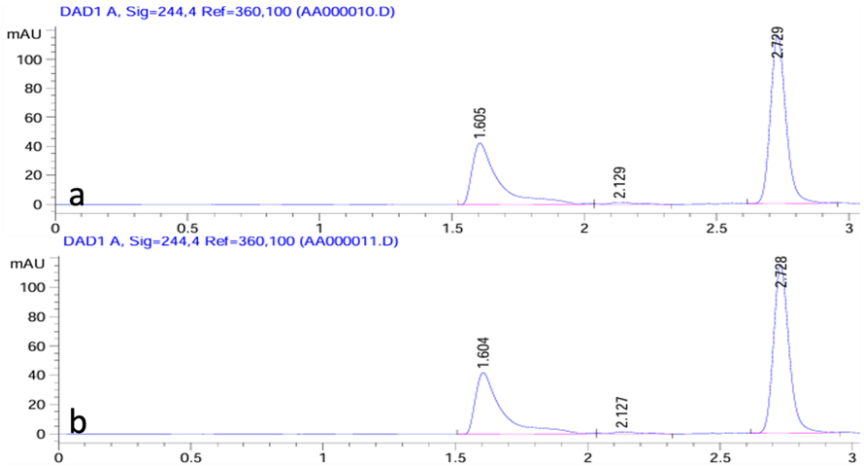

Fig 5. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Jonkheer van Tets juice

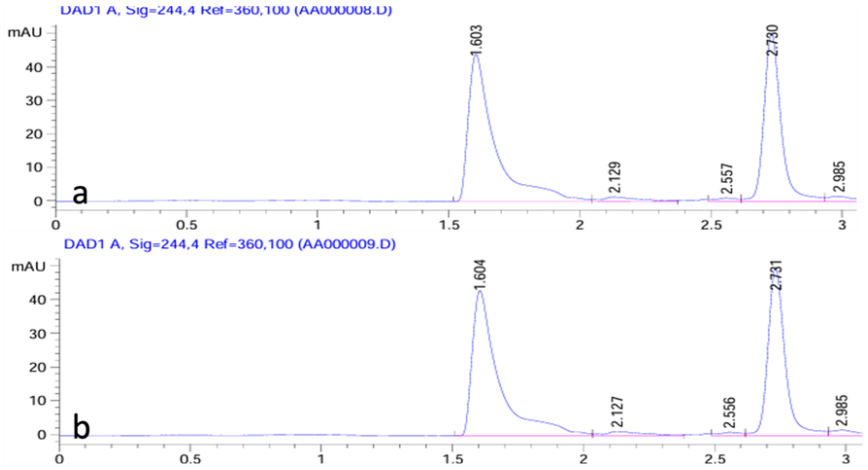

Fig 6. HPLC chromatogram of vitamin C before (a) and after reduction (b) in Rolan juice

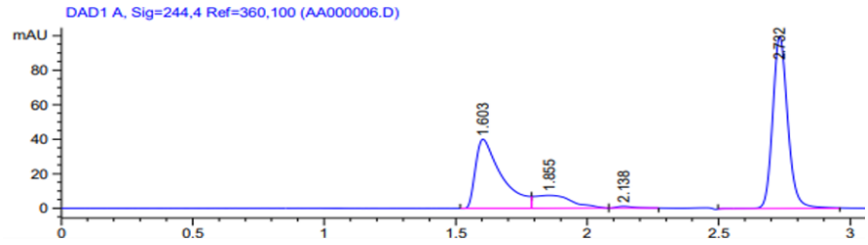

Fig 7. HPLC chromatogram of ascorbic acid standard (10 ppm)

The lowest vitamin C content had the juice of the Stanza variety. It was 3.21 ± 0.1 mg/100 g of juice before reduction (Figure 2a) and 6.23 ± 0.28 mg/100 g of juice after reduction (Figure 2b). HPLC chromatograms of all tested varieties before and after reduction are shown in Figures 1-6. Figure 7 shows the HPLC chromatogram of the ascorbic acid standard.

3.2. Antioxidant activity of red currant juices

The results of the antioxidant activity of the juices are shown in Table 2. The juice of the Redpoll variety showed the strongest free radical scavenging activity (IC_{50} = 1.76 ± 0.25 mg/mL), and the weakest expressed the juice of the Rolan variety (IC_{50} = 6.65 ± 0.84 mg/mL).

The best ability to inhibit lipid peroxidation also expressed Redpoll variety (IC_{50} = 2.52 ± 0.90 mg/mL), and the weakest expressed the juice of Stanza variety (IC_{50} = 6.23 ± 1.28 mg/mL).

Statistical analysis revealed that the antioxidant activity in the DPPH system differs between the juices of certain varieties of red currant and that the varieties Redpoll and Makosta are significantly different from each other, but also the other four tested varieties with a statistical significance of 95%.

4. DISCUSSION

This research shows that different varieties of red currants have different vitamin C content as well as distinct antioxidant activity. The amount of ascorbic acid in juices of 6 varieties of red currant ranged from 3.21 to 33.14 mg/100 g of juice, and from 6.23 to 66.52 mg/100 g of juice after the reduction of the samples. The juice of the Redpoll variety had the highest amount of ascorbic acid, and the juice of the Stanza variety had the lowest amount.

Examining the chemical composition of fresh fruits of 11 varieties of red currant, Djordjević et al. (2010), showed that the fruit of the Jonkheer variety had the lowest content of vitamin C (52.8 mg/100 g), and the fruit of the Rondom variety had the highest content (66.5 mg/100 g). The results of vitamin C content of fresh fruits of Rolan, Stanza, Redpoll and Makosta varieties were also in that range. The amount of vitamin C was lower in juices analyzed in this study compared to fresh fruits in Djordjević et al. (2010). A possible reason might be processing fruits into juices, during which the content of vitamin C decreases, due to the oxidation of ascorbic acid into dehy-

Table 2. Antioxidant activity of red currant juices (IC_{50} mg/mL)

Red currant juice sample	DPPH	β-carotene
Redpoll	1.76±0.25a	2.52±0.90a
Rolan	6.65±0.84b	3.96±0.92b
Jonkheer van Tets	6.44±0.90b	4.52±1.45c
Stanza	5.30±0.34b	6.23±1.28d
Rondom	5.10±0.43b	4.68±1.56c
Makosta	3.31±0.26c	4.46±1.44c

[a,b,c,d]Different letters in columns indicate a statistically significant difference between samples (Tukey test, $p<0.05$).

Results are presented as the mean value of three individual measurements ± standard deviation.

droascorbic acid (Kampuse et al., 2002). The oxidized form of vitamin C can be easily reduced to ascorbic acid under the influence of reducing agents, such as DTT. The results showed that the content of ascorbic acid was significantly higher after the reduction, and that during the processing of fruits into juices, a significant amount of vitamin C was oxidized. The highest increase in vitamin C content occurred in the juice of the Makosta variety, by 103.1%, and the least in the juice of the Stanza variety, by 94%, which shows that during the processing of fruits into juices, there was a partial loss of ascorbic acid content.

Podskalská et al. (2024) evaluated the vitamin C content in some red and black currants juices, and among them was Jonkheer. Although results were expressed as the mean value of several samples of red currants, ascorbic acid content was 24.1 mg/100 g. As the authors highlighted, these differences arise from climatic conditions, higher maturity at harvest, as well as the samples themselves.

Kidoń and Narasimhan (2022) analyzed the ascorbic acid content of Jonkheer van Tets juice obtained with different lengths of ultrasound treatments. The ascorbic acid content was 13.2 to 16.5 mg per 100 mL depending the time used for the ultrasound treatment, and the authors used DTT to measure complete vitamin C. Nevertheless, the vitamin C content was lower compared to our Jonkheer sample after reduction (23.52 mg/100 g) but higher than before the reduction (11.62 mg/100 g). Since the preparation and determination are almost the same, we can conclude that geographic position and climate might be essential parts for the ascorbic acid production in red currants.

Red currant shows antioxidant activity and has the potential to be a good source of antioxidants. The antioxidant activity of red currant is determined by phenolic compounds, mostly anthocyanins, and partly by ascorbic acid (Benvenuti et al., 2004). The antioxidant activity in this work was carried out by two methods. The DPPH method measures the ability of the sample to capture free radicals. The other one is the β-carotene bleaching assay that evaluates the capability of the sample to prevent loss of β-carotene in the β-carotene/linoleic acid model. The results showed that the DPPH antioxidant activity was in the range of 1.76 mg/mL (Redpoll variety) to 6.65 mg/mL (Roland variety). Djordjević et al. (2010) also determined the antioxidant activity of juices of 11 varieties of red currant using this method and it was in the range of 1.9 mg/mL (Redpoll variety) to 8.4 mg/mL (Jonkheer variety). The IC_{50} values obtained by DPPH method in our research were lower for the varieties Redpoll, Jonkheer, Stanza, Rondom and Makosta, which means they showed stronger antioxidant activity.

The differences in the antioxidant value between our research and the work of Djordjević et al. can also be explained by the different preparations of the samples. These samples were previously diluted with water, centrifuged and the supernatant was used for further analysis. Also, in this research, the samples were stored at a lower temperature.

The variety Rolan, which in our research showed the weakest antioxidant activity (6.65 mg/mL), in the work of Djordjević et al. (2010) showed better antioxidant activity (5.5 mg/mL) and it is ahead of the varieties Jonkheer, Stanza and Makosta.

Konić-Ristić et al. (2011) determined the antioxidant activity of the juices of 5 types of berries and for the red currant juice, they obtained the IC_{50} value = 3.14 mg/mL, although the variety used was not specified.

The antioxidant activity which measures the prevention of the β-carotene loss of these red currant juices, has not been determined before.

5. CONCLUSION

Red currants (*Ribes rubrum* L.) are widely grown and used in European countries and beyond, both fresh and processed. Although they are widely used, there is significantly less data on them compared to black currants. Thanks to favorable properties for cultivation and numerous potential and proven positive effects on health, red currant is becoming the subject of an increasing number of studies.

Examining the vitamin C content and antioxidant activity of the juices showed that the best results were obtained from the Redpoll variety juice. The data obtained in this work were in accordance with the already available results on the antioxidant activity of red currant juice but also indicate that the antioxidant potential varies to the variety of red currant. It was also established that processing into juices leads to a significant loss of vitamin C due to its oxidation. The results of this work can serve as an incentive for the use of red currant and its products in the diet, as well as for the selection of varieties with favorable nutritional characteristics.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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