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Short communication

Antioxidant potential and polyphenol content of five new cultivars of raspberries

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Introduction

During 2021 and 2022, the pandemic of corona viruses (COVID-19) caused the enormous increase in use of antioxidants and supplements. The latest recommendations from the World Health Organization were to use natural sources of antioxidants which can help in boosting immunity (WHO, 2019). Dietary antioxidants from fruits and vegetables play an important role in mitigating the damaging effects of reactive oxygen species (ROS) on cellular macromolecules (lipids, proteins, and DNA) (Rao et al., 2010). The raspberries have high antioxidant phytochemical contents which can be preserved by freeze-dried process. The yield of bioactive compounds from plant sources depends on solvent properties, nature of the plant material and extraction temperature and time (Xue et al., 2020). In the green chemistry approaches, natural deep eutectic solvents (NADES) were typically used as a substitute for organic solvents in order to preserve the environment (Espino et al., 2016).

Materials and methods

Plant material

Five new raspberry cultivars, non-commercially grown in Serbia in the system of integrated agricultural production (Adelita, Glen dee, Himbo top, San Rafael and Tula magic) were harvested from commercial plantation of the company Green hit. At the beginning of July 2021, berries were hand-harvested during the first year of vegetation. The samples were freeze-dried and stored at -20 °C until analysis.

Optimization of the antioxidant extraction process with the Natural Deep Eutectic Solvents

The NADES were selected based on literature data and prepared in ratio choline chloride and urea of 1:2 (Di Pietro et al., 2021). In NADES water was added in certain percentages (up to 45%). Approximately 100 mg of freeze-dried samples were extracted with 1.8 mL of NADES using ultrasonic bath for 20 minutes at 40 °C. Extracts were centrifuged at 13400 rpm for 5 min and supernatants were used for further analyses.

Determination of antioxidant activity

Radical scavenging capacity was measured using modified 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method (Ivanović et al., 2020). For each sample, 200 µL of the NADESs extracts were mixed with 1.8 mL of DPPH in methanol (0.04 mg/ml) and incubated 30 minutes in the dark. The antioxidant activity was presented as amount of the freeze-dried fruit in NADES which is required to scavenge 50% of the DPPH (IC 50) and it was determined using a nonlinear regression algorithm (Konić-Ristić et al., 2010). The procedure was repeated with 70% ethanol and acetone as controls.

Determination of total phenolics

The total phenolic content was estimated by Folin-Ciocalteu method (Ivanović et al., 2020). Freeze-dried fruits (1.5 g) were dissolved in 25 mL of 70 % (V/V) ethanol and extracted in ultrasonic bath during 45 minutes. Re-extraction was repeated in the same manner. For a

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colored reaction, 250 µL of each sample was added in 1.25 mL of 9:1 diluted Folin-Ciocalteu reagent and 1 mL of 7.5 % Na_2CO_3. After incubation (1 h) at room temperature, the absorbance at 765 nm was measured. Results are expressed as equivalents of gallic acid. The measurements were done in triplicate.

Results and discussion

In order to compare efficiency of the antioxidants extraction from raspberries, IC 50 values were determined for ethanol, acetone and NADES extracts. The best radical scavenging potential was established for NADES with 15% water, similar to acetone and better than ethanol extract (1.59, 1.56 and 2.33 mg/mL respectively). Further increase of water content in NADES provoked decrease radical scavenging capacity and increase of IC 50 values. The NADES with 15% water was used to determine the antioxidant potential of different raspberry cultivars in this study. The lowest IC 50 value was obtained for the San Rafael variety, followed by Himbo top, Tula magic, Glen dee and the highest for the Adelita variety (1.34, 1.44, 1.58, 1.70 and 2.86 mg/mL respectively). The same solvent (NADES with 15% water) was tested for determining of total polyphenols, however choline chloride reacted with Folin-Ciocalteu reagent so the standard procedure with ethanol was used for that purpose. The total concentration of polyphenols is expressed as equivalents of gallic acid (mg/g). The obtained results for different varieties were 14.07 (Glen dee), 14.04 (Himbo top), 12.03 (Tula magic), 9.88 (San Rafael) and 8.34 (Adelita). Comparing results from these two essays, it was concluded that results obtained from radical scavenging activity and total polyphenols experiments are not correlated. Although polyphenols are well known potent antioxidants they are not the only molecules in plants that possess this role. Nevertheless, Adelita variety showed the lowest polyphenol content and the highest antioxidant capacity. On the other hand, Himbo top variety showed the best overall results compared to the others. The San Rafael variety was practicaly interesting with the lowest IC 50 value despite the low polyphenol content. This result should be further investigated with detailed analysis of the chemical composition for this variety. For the remaining three varieties, similar results were obtained.

Conclusion

This study implemented green chemistry approach for determination of total polyphenols and antioxidant capacity of five new raspberry varieties cultivated in an integrated production system. The using of NADES for DPPH radical scavenging essay has been optimized. Obtained results for Adelita variety showed the weakest score while Himbo top variety the best on the performed tests. For the other varieties similar results as for Himbo top variety were obtained. These results confirmed that polyphenols were not the only ones responsible for antioxidant activity and that both essays should be performed for determination of the health benefits for different fruits and vegetables.

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