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Antioxidant capacity of blackcurrant (*Ribes nigrum* L.) leaves and buds

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Abstract. The antioxidant capacity is the combined free radical scavenging effect of all antioxidant compounds found in the studied system. There is a growing need for accurate, numerical determination of this capacity (for easier comparison), so there are many analytical procedures, methods, and measurement systems available to researchers. Neither one is able to model the totality of real, naturally occurring reactions; therefore, conclusions about the antioxidant power of the studied sample can be drawn only after using several methods. In this work, the total phenolic content (TPC) of blackcurrant leaves and buds was determined, and the antioxidant capacity was tested using the DPPH and FRAP assays. 80% methanol was the most effective in the extraction of phenolics followed by 80% ethanol, while for the antioxidant capacity the acetone (50%)/water/acetic acid (2%) mixture proved to be the best. Significant differences were observed between cultivars and sampling dates, but the pattern of variation during the harvest period was similar for all cultivars.

Keywords and phrases: polyphenols, extraction solvent, Ruben, Fertődi and Tisel cultivars

1. Introduction

Blackcurrant (*Ribes nigrum* L.) is a 1–2 m tall shrub, preferring the temperate climate. The crop is widely cultivated both for commercial and domestic use in the major part of Europe and Northern Asia as well as in Romania. The main goal

of blackcurrant growers is harvesting the berries rich in polyphenols and vitamin C, which are used in food and beverage manufacturing. The leaves and buds are also important raw materials for the food industry and cosmetics (*Ziemlewska et al.*, 2021). The leaves of the blackcurrant are simple, lobed, with 3–5 lobes; the 5–10 cm leaves are almost rounded and the leaf edge is irregularly double serrated. Blackcurrant was already popular in mediaeval German folk medicine for rheumatic complaints and as a diuretic. In modern medical terms, the leaves and fruit of the blackcurrant were used to treat patients with anuria. By the 18th century, it had become a popular herb in France, used to treat a wide range of ailments, including gout, joint pain, diarrhoea, and coughs. The health benefits of blackcurrant anthocyanins (BCA) suggested the potential for BCA use as a key ingredient of functional food or therapeutic product to treat or prevent various chronic diseases. Its antioxidant and anti-inflammatory activities have been widely studied and summarized by *Cao et al.* (2021). They protect against oxidative stress, neuron toxicity, and carcinogens. The phytoestrogenic activity also explains certain anti-aging effects of BCA. In their studies, *Nowak et al.* (2016) investigated the effects of blackcurrant leaf extract as an antioxidant and antimicrobial agent in vacuum-packed meat products.

The essential oil of blackcurrant leaves has been studied previously, and differences in the monoterpene hydrocarbon profile between different cultivars were reported (*Stevic et al.*, 2010). *Orav et al.* (2002) analysed the composition of blackcurrant aroma extracts from berries, leaves, and buds and reported that in the oils of the different blackcurrant materials the same substances, though with quantitative differences, were present.

Blackcurrant buds serve as a raw material for the preparation of essential oils and absolutes, which are used for flavouring in cosmetics and food products (*Orav et al.*, 2002). Buds of *R. nigrum* are pedicellate, ovoid, brown, towards the tip with glands, which are very well highlighted in the longitudinal section through them. They contain proanthocyanidines, flavonoids, phenolic acids, amino acids, catechin, enzymes, and vitamin C as well (*Chişe*, 2018).

Nowadays, great emphasis is placed on the study of plants with antioxidant properties. Many have studied the fruit of the blackcurrant and its berries (*Orav et al.*, 2002; *Tabart et al.*, 2006; *Teleszko & Wojdylo*, 2015), but the leaves and buds have received less attention from this point of view.

The antioxidant capacity of *R. nigrum* leaves and buds growing in the Harghita County region (Romania) has not been studied before, and no literature data is available on its antioxidant properties. Therefore, the aim of this study was to determine the total phenolic content and the antioxidant capacity of these two parts of the plants of different cultivars.

2. Materials and methods

2.1. Reagents and chemicals

Methanol (HPLC grade) and Folin–Ciocâlteu reagent (2 N) were purchased from Merck (Germany). Gallic acid (99% purity), L-ascorbic acid (reagent grade), anhydrous sodium carbonate (99% purity), iron (III)-chloride (sublimed grade, ≥ 99.9% trace metals basis), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, for the spectrophotometric determination of Fe, ≥ 99.0%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 90% purity) were purchased from Sigma-Aldrich (Germany).

2.2. Plant material

Three varieties of blackcurrant (*R. nigrum* L. Ruben, *R. nigrum L.* Fertődi, and *R. nigrum L.* Tisel) leaves and buds were harvested in the village of Sânmartin, Harghita County, in the spring and summer of 2021. Bud samples were collected on 6 February and 12 April, while the leaf samples were collected on 28 June from each cultivar. The leaves and buds were air dried in shade at 22 ˚C for ten days and then ground in electric grinder.

2.3. Sample preparation

Half gram of dried samples was extracted with 10 mL of the following extraction solvents:

– 80% (v/v) methanol,
– 80% (v/v) ethanol,
– 50% (v/v) acetone with 2% (v/v) acetic acid, and
– 70% (v/v) acetone with 2% (v/v) acetic acid (*Tabart et al.*, 2007).

The mixtures were shaken for 4 hours, centrifuged at 3,461 RCF for 10 minutes, and the supernatant was removed and used for further determinations. Each sample was independently extracted in triplicate, and analyses were performed the same day.

2.4. Total phenolics content (TPC)

Total phenolics were determined according to the Folin–Ciocâlteu method (*Singleton & Rossi*, 1965). This protocol is not very accurate for phenols, but it gives a good picture of the total phenol content. 50 µL of sample diluted with 200 µL of methanol: water (4:1) solution was mixed with 0.25 mL of Folin–Ciocâlteu reagent, and, 1 minute later, 1 mL of sodium carbonate (0.7 M) was added. The mixture

was heated to 50 °C and kept at this temperature for 5 minutes. After cooling, the absorbance was measured at 760 nm on a Varian Cary 50 UV spectrophotometer (Varian Co., USA). Gallic acid (Sigma) was used as standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (DW) of plant material. Measurements were performed in triplicate on each sample.

2.5. DPPH assay

The antioxidant capacity was measured in the methanolic extracts using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by *Nour et al.* (2014). Briefly, each methanol-diluted extract (50 µL) was mixed with 3 mL of DPPH methanolic solution with a concentration of 0.004% (v/v). The mixture was incubated for 30 minutes at room temperature in the dark, and the absorbance was measured at 517 nm on Varian Cary 50 UV–VIS spectrophotometer (Varian Co., USA).

The percentage of the DPPH consumed was calculated with the following equation:

$$E\% = 100 \cdot \frac{A_o - A_1}{A_o}, \quad (1)$$

where: A_o – the initial absorbance, and A_1 – the absorbance in the presence of the extract.

Measurements were performed in triplicate from all samples.

2.6. Ferric ion reducing/antioxidant power (FRAP) assay

The antioxidant potential of blackcurrant bud and leaf extracts was also determined using a FRAP assay measuring the change in absorbance at 593 nm due to the formation of a blue-coloured Fe^{2+}-tripyridyl-triazine compound from colourless oxidized Fe^{3+}-form by the action of electron-donating antioxidants (*Benzie & Strain*, 1996).

The stock solutions required for the FRAP solution were prepared as follows:

Acetate buffer: 3.1 g Na-acetate + 16 mL acetic acid + 1 L distilled water;

TPTZ solution: 0.312 g TPTZ + 100 mL distilled water + 336 µL HCl;

Ferric chloride solution: 0.54 g $FeCl_3$ + 100 mL distilled water.

The FRAP solution was prepared as follows:

25 mL acetate buffer + 2.5 mL; TPTZ solution + 2.5 mL ferric chloride solution.

The absorbance was measured spectrophotometrically at 593 nm with Varian Cary 50 UV–VIS spectrophotometer (Varian Co., USA). Calibration was performed with ascorbic acid, and results were expressed as milligrams of ascorbic acid equivalents (AsA) per gram of dry weight of plant material. Triplicate measurements were performed from all samples.

2.7. Statistical analysis

Data are expressed as the means ± standard deviation for at least three independent measurements. Statistical analysis was made using Microsoft Excel 2016. Significant differences ($p \leq 0.05$) between means were evaluated by ANOVA and *t*-test.

3. Results and discussions

Buds collected in early February were still dormant, smaller, and more closed than those collected in April. The buds collected in April were slightly swollen and had a strong scent. Leaves were between 5 and 7 cm long and varied in width from 3.5 to 4.5 cm.

In order to determine the antioxidant capacity and the total phenolic content of the samples, the responsible components must be first extracted from the plant material to exclude the possible interfering effects of other constituents during the analyses. Using the four different solvents, which were reported by *Tabart et al.* (2007), *Kowalski & de Mejia* (2021), and *Piotrowski et al.* (2016) to be the best ones to perform the extraction, the goal was to find the one that proves to be the most efficient in the analysis of the collected blackcurrant samples.

3.1. DPPH radical scavenging activity

The results obtained with this method showed that the antioxidant capacity of the bud and leaf extracts was almost 100%, so a tenfold dilution was made, and the measurements were repeated.

The inhibition percentage in the diluted bud extracts (*Figure 1*) appears to be higher in samples collected in February, but only to a very small extent compared to the results of the buds collected in April. As expected, based on literature data, the buds at rest had a higher DPPH radical scavenging activity than the already swollen, popping buds. Analysing the measurement data, it can be established that the DPPH radical scavenging activity of the samples collected from the Ruben cultivars in February did not differ significantly from the ones collected in April ($p > 0.05$).

The comparison of the efficiency of the solvents used for extraction led to the conclusion that the use of acetone-containing solutions is better than the alcoholic ones. This fact was also supported by the significance analysis too since the calculated *p*-value in this case was 0.05. However, comparing the efficiencies of solvents containing 50% (v/v) acetone and 70% (v/v) acetone, it was found that there was no significant difference between the two extraction solvents.

The DPPH assay results in the case of blackcurrant leaf extracts are presented in *Figure 2*. Notable differences were not observed for leaves as compared to buds; the inhibition % values were similar to those obtained for buds collected at different times despite the fact that the average values continue to decrease as the buds develop into leaves (from February to June).

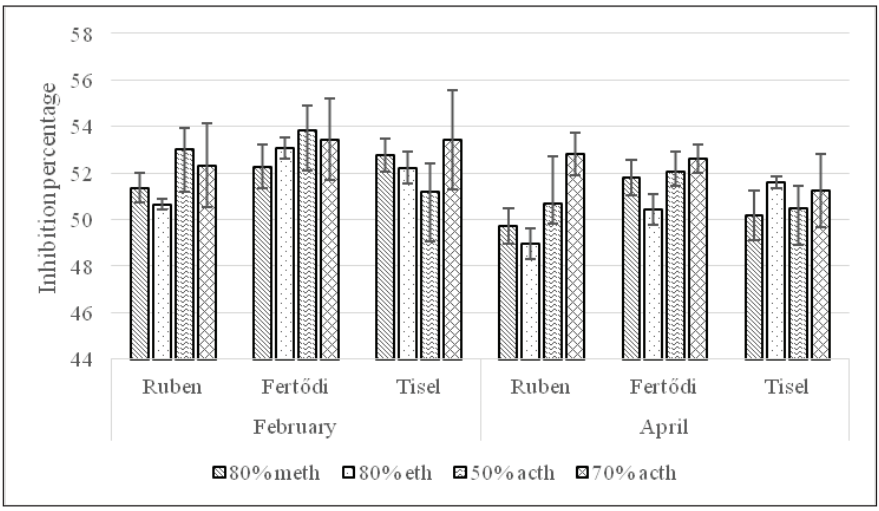


Figure 1. DPPH inhibition percentage in tenfold-diluted blackcurrant bud extracts

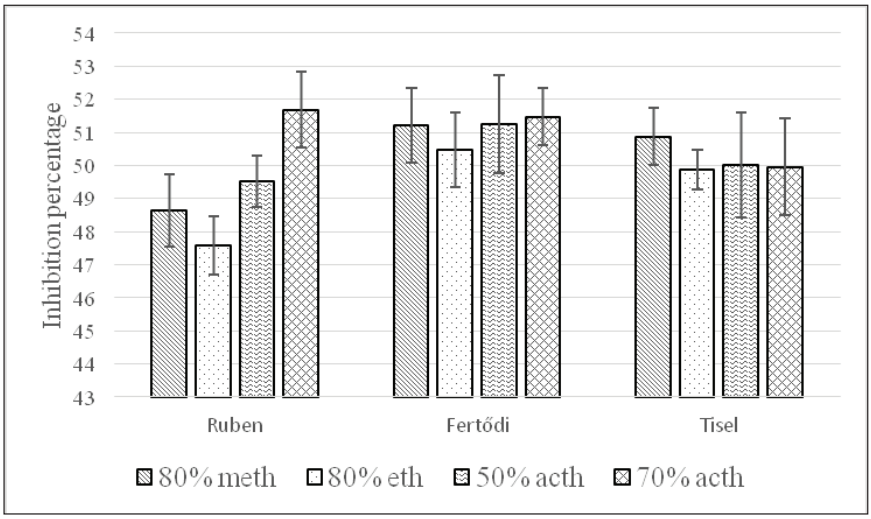


Figure 2. DPPH inhibition percentage in tenfold-diluted blackcurrant leaf extracts

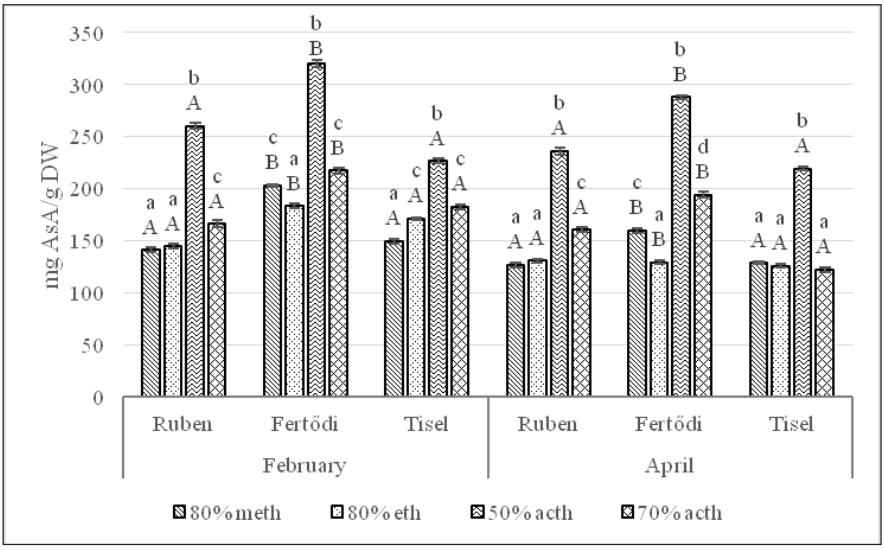
In this case, the significance analysis ($p > 0.05$) showed that the method of extraction (i.e. the quality of the solvent used) did not affect the results of the DPPH assay.

In conclusion, the DPPH inhibition percentage results obtained for leaves and buds were similar to the ones reported previously (*Bryan-Thomas*, 2016; *Krzepilko et al.*, 2018).

3.2. Antioxidant-reducing power (FRAP assay) of the extracts

The results of the FRAP assay led to the conclusion that a higher antioxidant capacity characterized the buds collected in February as compared to the buds collected in April (*Figure 3*). An 8.1% difference can be observed in the average antioxidant capacity in the case of Ruben, 16.4% in Fertődi, and 18.2% in Tisel.

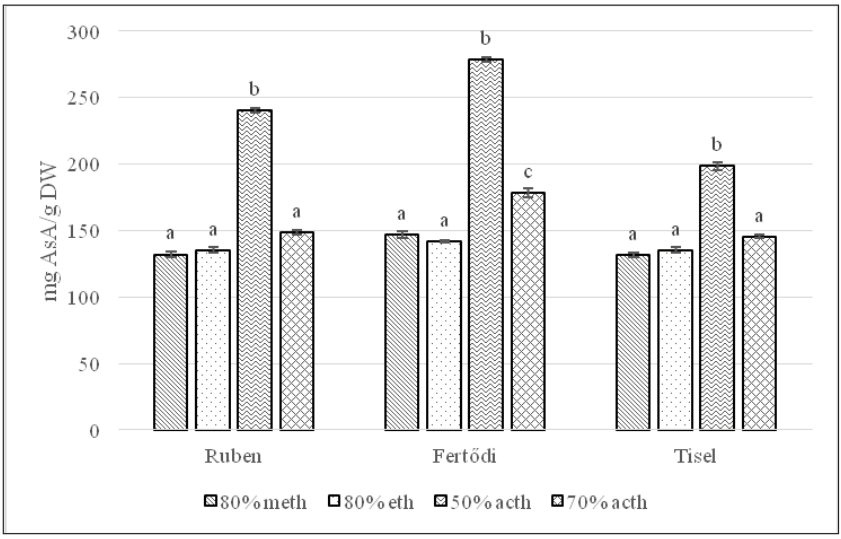
Comparing the cultivars' data, the highest antioxidant capacity was measured from the Fertődi samples. Furthermore, the acetone-containing extraction mixture had a 30% higher efficiency than the alcohol-based mixture, of which the mixture of 50% acetone and 2% acetic acid was found to be the most effective by 32%.



Notes: a–c significantly different between extraction solvents within cultivars; A–C significantly different between cultivars.

Figure 3. FRAP assay of blackcurrant bud extract in mg AsA/g DW

Fertődi shows the highest antioxidant capacity for leaves too (141–278 mg AsA/g DW), while the Ruben and Tisel samples show similar results (132–240 mg AsA/g DW) (*Figure 4*). Despite the difference, statistical analysis shows no significance in these values ($p > 0.05$).



Notes: a–c significantly different between extraction solvents within cultivars.

Figure 4. FRAP assay of blackcurrant leaves in mg AsA/g DW

A study published in 2007, which also tested the antioxidant capacity of blackcurrant leaves, found that leaves harvested in June had the highest antioxidant capacity. This is due to the development of the leaves (*Tabart et al.*, 2007).

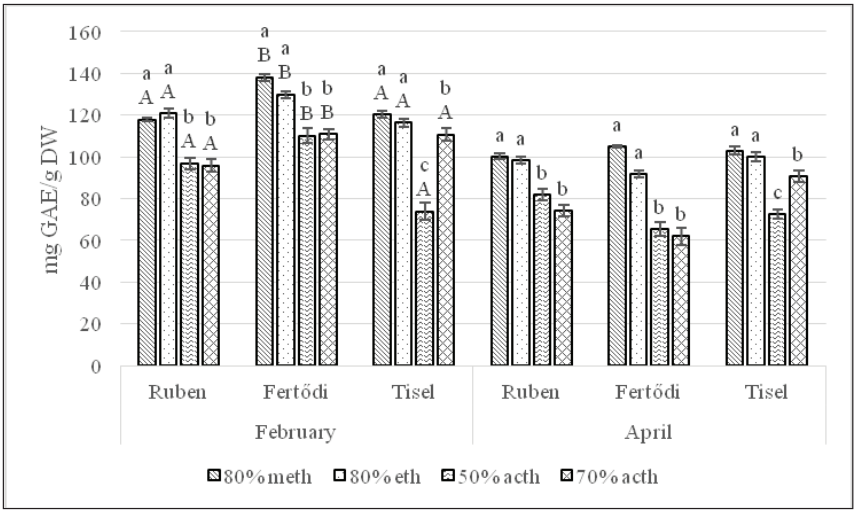
3.3. Total phenolic content

The polyphenolic components are responsible for the most significant antioxidant capacity (*Staszowszka-Karkut & Materska*, 2020). These components can be determined spectrophotometrically with the Folin–Ciocâlteu reagent, which is also an antioxidant capacity measurement with electron transfer (*Munteanu & Apetrei*, 2021; *Tabart et al.*, 2009). Various methods were tested to select the best extraction procedure expressed as total phenolics (TP) in buds and leaves. High yields of phenolic compounds were obtained from both buds and leaves with a solvent containing 50% (v/v) ethanol as compared to 30% (v/v) and 70% (v/v) ethanol and an extraction time of 15 min together with an ultrasonic bath. It was also observed that the TP content was higher in the leaves (89–97 mg GAE/g DW), with no significant difference ($p > 0.05$) between the treatments, than in the buds (45–56 mg GAE/g DW), with significant difference ($p < 0.05$) between the treatments (*Vagiri et al.*, 2012). *Tabart et al.* (2007) analysed the blackcurrant cultivar 'Noir de Bourgogne' and measured the total polyphenol content of 46.0 mg/g and 45.1 mg/g chlorogenic acid-equivalent (CAE) fresh weight (FW) in leaves and buds, respectively, using acetone/acetic acid/water (70:28:2) solvent. Previous

work has shown that the best extraction of phenolic compounds from blackcurrant buds and leaves is achieved with the acetone/acetic acid/water mixture.

In contrast, a higher total phenol content in buds was measured as compared to leaves, and the obtained TP values were higher with alcohols than with acetone/acetic acid/water solvents (*Figures 5–6*).

Phytochemical research shows that blackcurrant leaves and buds are a valuable source of polyphenols. The polyphenol content of leaves and buds can be up to five times higher than the polyphenol content of the fruit (*Tabart et al.*, 2006). The composition of the blackcurrant leaves has been described by several authors such as *Cortez & Gonzales de Mejia* (2019), *Ferlemi & Lamari* (2016). They identified mainly flavones and phenolic acids. Depending on the extraction conditions (pH, solvent type), the polyphenol content of the leaves was estimated to be between 4,000 and 20,000 mg/100 g DW (*Tabart et al.*, 2007). The values obtained in this study are situated in the middle of this range.

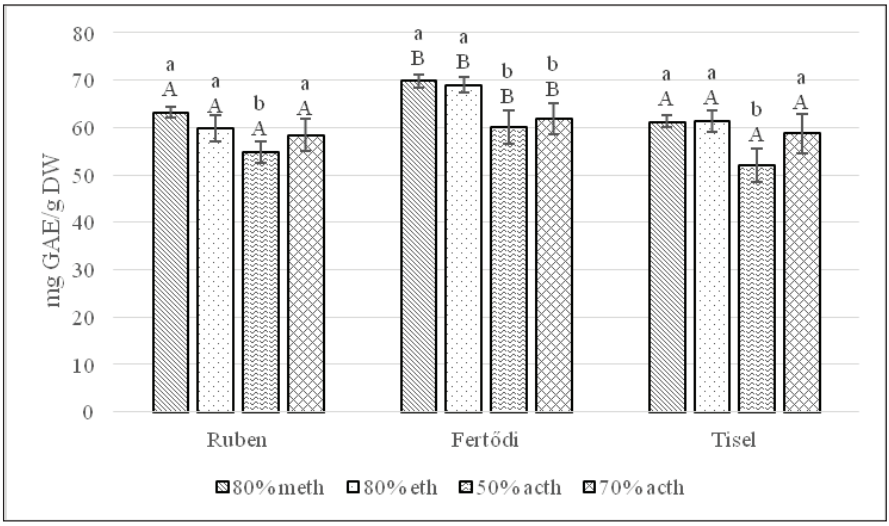


Notes: a–c significantly different between extraction solvents within cultivars; A–B significantly different between cultivars.

Figure 5. The total phenolic content of *R. nigrum* buds in mg GAE/g DW

Significant difference was observed in the total polyphenol content measured in Fertődi cultivars collected in February. No significant differences were noted in total polyphenol content measured in the buds of the different cultivars, collected in April. Nonetheless, a significantly ($p < 0.05$) – 33.7% – higher TP content was measured in the buds collected in February as compared to the ones collected in April in the case of Fertődi cultivars. Among the three blackcurrant cultivars, Fertődi buds had the highest total polyphenol content, with 110–138 mg GAE/g

DW polyphenol content measured in buds collected in February, depending on the solvent. Similar to the FRAP antioxidant capacity, the total polyphenol content of the buds was lower in samples collected in April.



Notes: a–b significantly different between extraction solvents within cultivars; A–B significantly different between cultivars.

Figure 6. The total phenolic content of *R. nigrum* leaves in mg GAE/g DW

The results of the leaf extracts also showed that the total polyphenol content was higher in Fertődi, which is in agreement with the values reported in the literature (*Koczka et al.*, 2018). Furthermore, the alcohol-containing solvent had a significantly higher (26% in buds and 10% in leaves; $p < 0.05$ and $p < 0.05$ respectively) result in the extraction than in the acetone-containing solvent.

Besides the nature of the solvent, phenolic content depends on other extraction parameters too such as pH, temperature, solvent to solid ratio, particle size, and shape of the sample (*Tabart et al.*, 2007).

4. Conclusions

As the previously reported data shows, little attention has been given to research on the biologically active substances of leaves and buds of different *Ribes nigrum* cultivars, which were the research material in this study.

Volatile solvents affect the accuracy of the measurements, as the standard deviation was the highest for acetone in all tests due to its volatility. The correlation study shows that polyphenols contribute significantly to the binding of the DPPH radical

and the reduction of Fe^{3+} ions. The values obtained are in agreement with literature data, and it can be concluded that blackcurrant buds and leaves are an excellent source of antioxidants. It can also be concluded that not only the blackcurrant fruit but also the buds and leaves contain beneficial phenolic compounds and can be utilized to produce functional, health-protective products. The results show that in order to obtain the optimal content of the desired bioactive compounds, it is important to take into account the genotype, the place of cultivation, the time of harvest, the stage of bud development, and the position of the leaves. Of the different solvents, the acetone (50%)/water/acetic acid (2%) mixture proved to be the best for obtaining extracts with higher antioxidant capacities in blackcurrant leaves and buds. For extracting phenolic compounds, we recommend solutions containing 80% ethanol or methanol. In terms of harvesting time, February proved to be better for buds. Although there were significant differences in phenolic compound content and antioxidant activity between the cultivars studied, the pattern of change during the growing season was quite similar.

Therefore, these natural substances could be added to products in order to prevent or delay their deterioration by action of oxygen in the air.

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Some aspects of plate number estimation of plate heat exchangers (PHEs). A case study

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Abstract. For the proper estimation of the plate number (N) of a plate heat exchanger (PHE) – in addition to the flow rates and thermophysical properties of fluids –, an appropriate correlation is needed for convective heat transfer coefficient (α) calculation. When one does not have a criterial equation for the corresponding plate shape, we propose a selecting method for α. With the suggested relationships from literature, we calculate the plate number of a geometrically known, similar heat duty PHE and choose those relationships that give the same plate number with the known heat exchanger. In our case study, the plate number determined by any of the screened equations for whole milk preheating has almost the same value ($n = 10 \pm 1$) regardless of the method used to solve the PHE model (plate efficiency and $N_{converg}$ or $K_{converg}$ convergence methods). For liquids' thermophysical property estimation, we recommend averaging the values given by equations from literature, followed by equation fitting.

Keywords and phrases: milk, thermophysical properties, plate heat exchangers, plate number estimation

1. Introduction

The main heat exchanger type in food industry is the plate heat exchanger (PHE), used for the heating or cooling of media with good rheological properties and with low solid content, to avoid solid deposition on the surface (usually Newtonian liquids as milk, various fruit juices, high-temperature cooking oils, cleaning and process waters, etc.) (*Mariott*, 1979; *Stoica et al.*, 2007; *Singh & Heldman*, 2013; *Kakaç et al.*, 2020). Hot water or steam (heating medium), chilled water, cooling water, brine or propylene glycol water solutions (cooling medium) are used as thermal agent (*Macovei*, 2001; *Kakaç & Liu*, 2002; *Heldman*, 2007; *Thulukkanam*, 2013). The PHE's operation is characterized by a continuous steady state (*Shah & Sekulić*, 2002; *Singh & Heldman*, 2013; *Roetzel et al.*, 2020), but it can also