

Technical paper

# The Effect of Ultrasonic Pretreatment and Sample Preparation on the Extraction Yield of Antioxidant Compounds and Activity of Black Currant Fruits

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## Abstract

The aim of the study was to evaluate the efficiency of an ultrasonic pretreatment at different amplitudes and extraction times, on the content of antioxidant compounds (phenolics, anthocyanins, ascorbic acid) and total antioxidant capacity of black currant fruits. Additionally, the influence of sample preparation (frozen storage/drying) was evaluated.

Extraction was performed in 60% ethanol with 0.15% HCl at a solvent-to-sample ratio of 15/1. Our results show that the ultrasonic pretreatment proved particularly useful for the recovery of high amounts of total anthocyanins in freeze-dried samples, ascorbic acid in frozen and oven air-dried samples, and total antioxidant capacity in freeze-dried and oven air-dried samples. The total phenolics content was not significantly influenced by the ultrasonic pretreatment. Freeze-drying increased the content of targeted compounds, to a very high significant level ( $p < 0.001$ ) for anthocyanins and ascorbic acid. Oven air-drying at 45 °C drastically decreased the ascorbic acid content.

Ultrasonication enhanced the extraction yield of black currant valuable compounds in strong relation to the operating conditions.

**Keywords:** Black currant, ultrasound-assisted extraction, phenolics, anthocyanins, ascorbic acid, FRAP

## 1. Introduction

For a long time, higher plants have been considered the richest sources of pharmacologically active compounds, in particular »phytochemicals« which have been extracted for their disease-preventing benefits. Phytochemicals are chemical compounds of secondary metabolic origin with high structural variability, belonging to isoprenoids, phenylpropanoids and alkaloids classes.

Within the *Grossulariaceae* family, black currants (*Ribes nigrum* L.) are species rich in phytochemicals, in particular flavonoids, anthocyanins and ascorbic acid, consumed either as such or in processed foods (jam, jelly, syrup, soft and alcoholic drinks). The major part of these bioactives display health beneficial properties based on their free-radical scavenging and antioxidant properties.<sup>1-2</sup>

In order to achieve high amounts of phytochemicals from these fruits and optimal preservation of their bioacti-

vity, proper extraction methods and minimal processing technologies are required. These issues become important also for the structure-activity relationship (SAR) studies and for the optimization of mixed extracts composition to be used in pharmaceutical, food or cosmetic industry.

Several conventional, non-conventional and hybrid new extraction technologies of phytochemicals have been described in the literature, finally leading to either an enriched crude extract or to a further purified extract.<sup>3</sup> The optimization of extraction parameters (solvent type, solvent concentration, solvent-to-solid ratio, temperature, time, other variables) is an important step to be considered either to obtain the highest extraction yield of targeted compounds or to achieve the lowest energy consumption.

Usually, individual extracts of compounds of interest are prepared in relation to optimal extraction of such compounds. Solvent extraction of anthocyanins is usually carried out in acetone, methanol, ethanol, water or combi-

nations or acidified methanol/ethanol solutions (up to 0.1% HCl) in order to obtain the red stable flavylum cation without significant degradation of acylated anthocyanins.<sup>4–6</sup> Solvent extraction of ascorbic acid is performed in an acidic environment, usually with 5% metaphosphoric acid.<sup>7–8</sup> However the optimum extraction conditions for valuable compounds depends on the type of plant material and on its chemical composition and must be individually designed.

Ultrasound-assisted extraction of various plant compounds (carbohydrates, phenolics, flavonoids, steroids, essential oils, phospholipids, etc.) became very attractive in particular because of the potential industrial application,<sup>9–10</sup> being considered a promising tool for an efficiently and energy saving extractive method. It was shown that ultrasounds act very efficiently during extraction due to an improved mechanical effect and by producing acoustic cavitations in the solvent, in relation to optimized operating conditions.<sup>11–12</sup> Other modern extraction techniques, such as pressurized liquid extraction, supercritical fluid extraction and microwave-assisted extraction were applied for various phytochemicals,<sup>13</sup> but with modest success for anthocyanins,<sup>14–16</sup> as these are thermolabile water soluble compounds.

The aim of this study was to evaluate the efficiency of an ultrasonic pretreatment at different operating conditions (amplitude and extraction time) on the content of antioxidant compounds (phenolics, anthocyanins, ascorbic acid) and on the total antioxidant activity of black currant fruits. Additionally, the influence of sample preparation (frozen storage, freeze-drying, oven air-drying) was evaluated in relation to the content of targeted bioactives extracted under the same conditions. The obtained crude extracts may find further application, in particular in food and pharmaceutical products, based on the synergistic effects exerted by all bioactive compounds.

## 2. Experimental

### 2.1. Sample Preparation and Experimental Setup

Commercially frozen black currant fruits (*Ribes nigrum* L.) were used for the experiments. Three types of processed samples were studied: (i) frozen (no treatment of acquired fruits) initially thawed at room temperature in dark; (ii) freeze-dried; and (iii) oven air-dried.

Chemical reagents of analytical grade without further purification were used.

#### 2.1.1. Drying Procedures

Freeze-drying was performed using a lyophilizer (Alpha 1-4 LD plus, Christ, Germany) at  $-50\text{ }^{\circ}\text{C}$  and 0.04 mbar, until 5% moisture was reached.

Oven air-drying was performed using a convection oven (UFE 400 with forced air circulation, Memmert, Germany) at  $45\text{ }^{\circ}\text{C}$  and a fan speed of 100%, until 6.115% moisture was reached.

### 2.1.2. Extraction procedures

#### Conventional extraction

Reducing sample size of plant material by grinding was performed before extraction. All types of samples were milled with a knife mill (Grindomix GM 200, Retsch, Germany).

As literature reports a maximum extraction of total anthocyanins and phenolics from black currants with 60% ethanol at a solvent-to-solid ratio of 19/1,<sup>17</sup> and considering that acidified aqueous ethanol increase extraction not only of anthocyanins (usually 0.1%) but particularly of ascorbic acid (usually 0.36%), we have used 60% ethanol with 0.15% HCl to prepare extracts for determination of total phenolics, total anthocyanins, ascorbic acid and total antioxidant capacity by FRAP, at a solvent-to-solid ratio of 15/1 (mL/g) in all runs.

Milled samples were subjected to extraction of antioxidant compounds. Samples were dispersed in the extraction solvent, stirred at 1100 rpm for 10 min using the magnetic stirrer (C-MAG HS7 IKAMAG, Ika, Germany) and then left for 50 min at  $4\text{ }^{\circ}\text{C}$ . This conventional extraction procedure was applied alone and together with ultrasonic pretreatment of the solvent/sample mixture.

#### Ultrasound-assisted extraction

The ultrasound-assisted extraction was performed using an ultrasonic device (Sonifier SLPe-150, Branson, USA) of 150 W power and 40 kHz frequency, equipped with a transducer to be dipped directly into sample. The solvent-sample mixtures were continuously irradiated for three predetermined extraction times (3, 6 and 10 min) and ultrasonic amplitudes (10, 40 and 70%). The room temperature in the water bath where vials contain

**Table 1:** Experimental data with different combinations of ultrasonic extraction time and amplitude in the extraction process.

Experiment run	Ultrasonic extraction time (min)	Ultrasonic amplitude (%)
E1	3	10
E2	3	40
E3	3	70
E4	6	10
E5	6	40
E6	6	70
E7	10	10
E8	10	40
E9	10	70
E10*	–	–

\*E10 = conventional extraction without ultrasonic pretreatment

ning the mixture were immersed was maintained by ice addition.

The experimental design for the extraction process applied to the three types of samples (frozen, freeze-dried and oven air-dried) is shown in Table 1.

After extraction, all samples were centrifuged at 8000 rpm at 4 °C for 10 min using the refrigerated centrifuge (Universal 320, Hettich, Germany). The obtained crude extracts were used for the determination of total phenolics, total anthocyanins, ascorbic acid and total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay.

## 2. 2. Determination of Moisture and Total Soluble Solids

Moisture content of samples (frozen, freeze-dried and oven air-dried) was determined at 105 °C using the moisture analyzer (ML-50, A&D Co. Ltd., Japan). Total soluble solids (TSS) of the fruit juice obtained by manually pressing, was determined by refractometry using an Abbe refractometer (AR2008, Krüss, Germany) at standardized temperature (21 °C). Values are expressed as refractometric TSS (°Brix).

## 2. 3. Total Phenolics Assay

The total phenolics content was determined according to the Folin-Ciocalteu spectrophotometric method.<sup>18</sup> The absorbance at 745 nm was measured. The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena, Germany) was used. Gallic acid was used as standard for the calibration curve. The content of total phenolics was expressed in milligram of gallic acid equivalents per 100 g dry mass (mg GAE 100g<sup>-1</sup> DM).

## 2. 4. Total Anthocyanins Assay

The content of total anthocyanins was determined spectrophotometrically by the pH differential method.<sup>4</sup> The Specord 200Plus UV-Vis spectrophotometer (Analytik Jena) was used. The content of total anthocyanins was expressed as milligram cyanidin-3-O-glucoside (Cyn-3-O-G) equivalents per 100 g dry mass (mg 100g<sup>-1</sup> DM).

## 2. 5. Ascorbic Acid Determination

Ascorbic acid content was determined by reversed-phase HPLC on a Knauer Smartline system equipped with UV detector, using a Nucleosil EC 250/4 100-5 C18 HD column and a gradient of 0.1% formic acid and acetonitrile. The column temperature was kept at 30 °C and the flow rate at 2.5 mL min<sup>-1</sup>. Detection was carried out at 245 nm. The content of ascorbic acid was expressed as milligram ascorbic acid per 100 g dry mass (mg 100<sup>-1</sup> DM).

## 2. 6. Total Antioxidant Capacity by Ferric Reducing Antioxidant Power (FRAP) Assay

The total antioxidant capacity was determined by the ferric reducing ability assay described by Benzie and Strain.<sup>19</sup> The absorbance of the mixture of anthocyanin extracts and FRAP reagent was measured at 593 nm after 5 min. The results were expressed as milligram ascorbic acid per 100 g dry mass (mg 100<sup>-1</sup> DM).

## 2. 7. Statistical Analysis

Data processing consisted in mathematical and statistical methods performed by “IBM SPSS 21.0” software, following hypothesis testing and correlation between variables by calculating the Pearson correlation coefficient  $r$  ( $r = \pm 1$  means perfect correlation), at a significance level of risk  $\alpha \leq 5\%$  and probability  $P \geq 95\%$ .

## 3. Results and Discussion

The extraction yield of antioxidant compounds – phenolics, anthocyanins, ascorbic acid – and the total antioxidant capacity of black currant fruits in relation to ultrasonic pretreatment conditions has been addressed, as such compounds are highly studied for the production of functional foods.

In the present study, black currant fruits (*Ribes nigrum* L.) characterized by 17.0°Brix of total soluble solids and 66% moisture were selected to prepare valuable extracts.

It is known that the type of extraction highly influences the quality of the final product to be used either as food supplement or ingredient.

The influence of conventional extraction hereby named control (noted as experiment E10 in Table 1), alone and combined with an ultrasonic pretreatment (noted as experiments E1–E9 in Table 1) on the recovery of high amounts of targeted antioxidant compounds (total phenolics, total anthocyanins, ascorbic acid, total antioxidant capacity) was studied. The environmentally friendly acidified aqueous ethanol (60% ethanol with 0.15% HCl v/v) and the solvent-to-sample ratio of 15/1 were used in all experimental runs as appropriate to extract simultaneously the antioxidant compounds of interest. Addition of hydrochloric acid is essential to extract ascorbic acid and to stabilize anthocyanins in the form of flavylium cation, but excess may lead to partial hydrolysis of glycosidic bond or cause breaking linkages with metals or co-pigments. Therefore, extraction was mainly conducted at 4 °C to minimize targeted bioactives degradation, in particular anthocyanins. Hydrochloric acid is preferred to organic acids which may lead to anthocyanin acylation. Diluted hydrochloric acid in ethanol does not significantly affect the extracts for the purpose of food application,

as finally small amounts of residues will be found. Despite the decline of clinical applications of diluted hydrochloric acid, historically it was described in various pharmacopoeias as gastric acidifier to assist food digestion or to reverse metabolic alkalosis.<sup>20</sup>

In our previously published work,<sup>21</sup> we found that ultrasound-assisted extraction of total anthocyanins and total antioxidant capacity of blackberries and sweet cherries determined an efficient recovery of these bioactives at a solvent-to-sample ratio of 15/1 and short extraction time, using vials immersed in an ultrasonic bath.

In the present study, we have extended our investigation to harsher ultrasonic applied conditions by using a device with a transducer and probe directly dipped into the solvent/sample mixture, which will improve the mass transfer and cell disruption. Two process variables – ultrasonic extraction time (3, 6 and 10 min) and ultrasonic amplitude (10, 40 and 70%) – have been considered as most critical for reproducing sonication results.

In addition to these, as sample preparation may influence to a greater extent the extraction yield, frozen, freeze-dried and oven air-dried black currant fruits were evaluated for the extraction efficiency.

The concentration of the selected antioxidant compounds and activity was determined on a dry mass basis for homogeneity.

The obtained results are indicated in Figures 1–4. For the simultaneous extraction of the antioxidant compounds, different optimized parameters were obtained for each class of compounds.

### 3. 1. Effect of the Ultrasonic Pretreatment on the Total Phenolics Content

The total phenolics content increased by approximately 4% compared to control when ultrasonic pretreatment (40 kHz frequency, 60% ethanol with 0.15% HCl, sol-

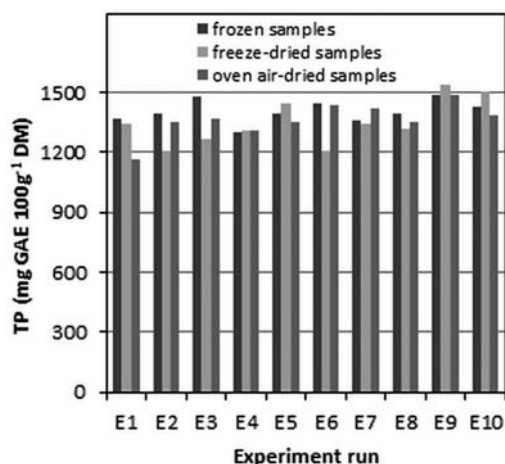


Fig. 1. Total phenolics content in black currant fruits (frozen, freeze-dried and oven air-dried) according to different ultrasonic conditions; significance of experimental runs (E1-E10) is indicated in Table 1.

vent-to-sample ratio 15/1, ultrasonic probe) was applied for 10 min at high amplitude (70%) in all three cases of sample preparation (frozen, freeze drying, oven air-drying), as presented in Fig. 1. The total phenolics content increased with the extraction time and amplitude. Statistical analysis indicates medium positive correlations of the total phenolics content to the ultrasonic extraction time ( $r = 0.393$ ,  $p < 0.05$ ), and to the ultrasonic amplitude ( $r = 0.405$ ,  $p < 0.05$ ), respectively. No statistically significant differences were found between the total phenolics content and the type of sample preparation.

### 3. 2. Effect of the Ultrasonic Pretreatment on the Total Anthocyanins Content

Results are presented in Fig. 2.

Considering the extraction of total anthocyanins (40 kHz frequency, 60% ethanol with 0.15% HCl, solvent-to-sample ratio 15/1, ultrasonic probe) the optimized ultrasound-assisted extraction conditions are as following:

- for frozen samples, 3 min extraction time at 70% amplitude, which determined an increase by approximately 4% (793.426 mg 100g<sup>-1</sup> DM) compared to control;
- for freeze-dried samples, 10 min extraction time at 70% amplitude, which determined an increase by approximately 20% (1041.318 mg 100g<sup>-1</sup> DM) compared to control;
- for oven air-dried samples, 6 min extraction time at 70% amplitude, which determined an increase by approximately 7% (847.85 mg 100g<sup>-1</sup> DM) compared to control.

High amplitude seems to favor the increase of the total anthocyanins content, but not of statistical significance. No statistical correlation was found between the ultrasonic extraction time and the total anthocyanins content.

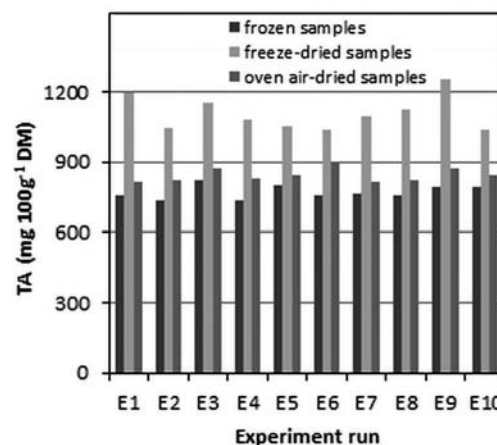


Fig. 2. Total anthocyanins content in black currant fruits (frozen, freeze-dried and oven air-dried) according to different ultrasonic conditions; significance of experimental runs (E1-E10) is indicated in Table 1.

Freeze-drying significantly increased the total anthocyanins content ( $p < 0.001$ ) compared to frozen storage and oven air-drying.

### 3. 3. Effect of the Ultrasonic Pretreatment on the Ascorbic Acid Content

Results are shown in Fig. 3.

Considering the extraction of the ascorbic acid (40 kHz frequency, 60% ethanol with 0.15% HCl, solvent-to-sample ratio 15/1, ultrasonic probe) the optimized ultrasound-assisted extraction conditions are as following:

- for frozen samples, 3 min extraction time at 10% amplitude, which determined an increase by approximately 50% (100.138 mg  $100\text{g}^{-1}$  DM) compared to control;
- for freeze-dried samples, 3 min extraction time at 40% amplitude, which determined an increase by approximately 7% (437.5 mg  $100\text{g}^{-1}$  DM) compared to control;
- for oven air-dried samples, 10 min extraction time at 10% amplitude, which determined an increase by approximately 50% (27.194 mg  $100\text{g}^{-1}$  DM) compared to control.

The influence of the ultrasonic extraction time and amplitude on the ascorbic acid content was not statistically significant.

Freeze-drying significantly increased the ascorbic acid content ( $p < 0.001$ ) compared to frozen storage and oven air-drying. The ascorbic acid content drastically decreased in oven air-dried samples.

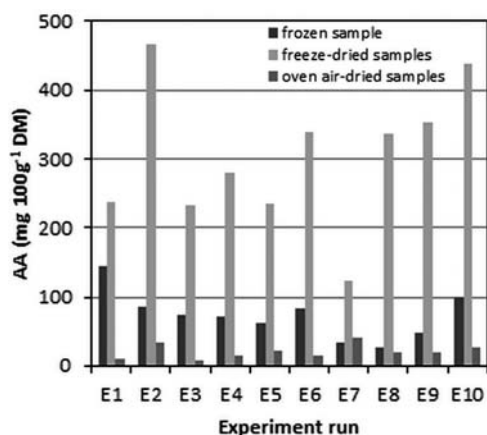


Fig. 3. Ascorbic acid content in black currant fruits (frozen, freeze-dried and oven air-dried) according to different ultrasonic conditions; significance of experimental runs (E1-E10) is indicated in Table 1.

### 3. 3. Effect of the Ultrasonic Pretreatment on the Total Antioxidant Capacity

Results are presented in Fig. 4.

Considering the total antioxidant capacity as measured by FRAP method (40 kHz frequency, 60% ethanol

with 0.15% HCl, solvent-to-sample ratio 15/1, ultrasonic probe) the optimized ultrasound-assisted extraction conditions are as following:

- for frozen samples, 6 min extraction time at 40% amplitude, which determined an increase by approximately 1% (2928.774 mg ascorbic acid  $100\text{g}^{-1}$  DM) compared to control;
- for freeze-dried samples, 10 min extraction time at 70% amplitude, which determined an increase by approximately 28% (3141.475 mg ascorbic acid  $100\text{g}^{-1}$  DM) compared to control;
- for oven air-dried samples, 3 min extraction time at 70% amplitude, which determined an increase by approximately 144% (2054.985 mg ascorbic acid  $100\text{g}^{-1}$  DM) compared to control; sometimes, the increase of the total antioxidant capacity with increasing temperature of applied treatment might be observed as in this case, probably due to the presence of other antioxidant intermediate compounds generated through the process, which were efficiently extracted under these ultrasonic conditions.

High amplitude seems to favor the increase of the total antioxidant capacity (low positive correlation).

The extracts prepared from freeze-dried samples showed the highest mean value of the total antioxidant capacity, while the extracts from oven air-dried samples showed the lowest.

Considering freeze-drying as the optimum sample preparation method to achieve efficient extraction (either conventional or ultrasound-assisted), we explored the relationship between the total antioxidant capacity and the targeted compounds. We found a good Pearson correlation ( $r = 0.682$ ,  $p < 0.05$ ) between the total antioxidant capacity and the total phenolics content. The correlations of the total antioxidant capacity to the total anthocyanins content, and to the ascorbic acid content, respectively, we-

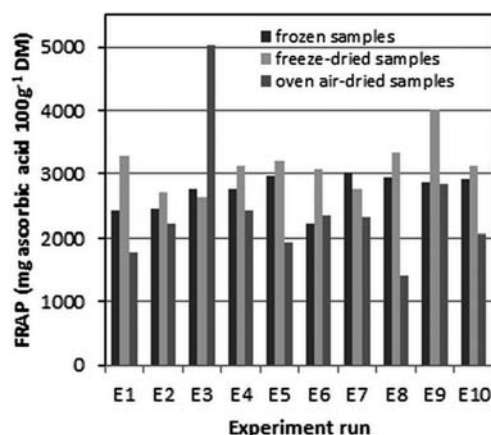


Fig. 4. Total antioxidant capacity as measured by FRAP method in black currant fruits (frozen, freeze-dried and oven air-dried) according to different ultrasonic conditions; significance of experimental runs (E1-E10) is indicated in Table 1.

re not statistically significant. Such results are in agreement to the other reported.<sup>22–23</sup>

Very high significant differences were found between the preparation type of samples and the content of total anthocyanins and ascorbic acid ( $p < 0.001$ ). Freeze-drying determined better preservation of bioactives content and a better extraction efficiency, the latter related to the particle size reduction. Oven air-drying at 45 °C didn't preserved efficiently the bioactives content, but showed efficient extraction influenced by the small particle size. This is particularly important when food processing (drying, preservation, storage) is employed which may lead to the loss of bioactive compounds.

We found high positive Pearson correlation between the total anthocyanins content and the ascorbic acid content ( $r = 0.780$ ,  $p < 0.001$ ), and good Pearson correlation between the total antioxidant capacity and the total anthocyanins content ( $0.426$ ,  $p < 0.05$ ) in all ultrasonic extracts.

The optimal ultrasound-assisted extraction conditions of amplitude and extraction time for high recovery of antioxidant compounds may vary between different studies due to different operating conditions, type of device, and not at least plant material or bioactive compounds. While literature is abundant in studies regarding ultrasound-assisted extraction of anthocyanins from by-products of black currant (pomace, press-residue), it is scarce in studies on black currant fruits. Some authors reported the solvent concentration effect on the content of polyphenols extracted from black currant fruits only by applying fixed conditions of ultrasound-assisted extraction performed with an ultrasonic bath of high frequency (100 kHz) for 30 min.<sup>24</sup> It becomes quite difficult to compare our results as the ultrasonic power and sample drying methods were not specified in the above mentioned work. Usually, when using ultrasound-assisted extraction, higher frequencies ( $>40$  kHz) and higher extraction time ( $>30$  min) decrease the extraction yield compared to conventional extraction.<sup>10</sup>

In another work, ultrasound-assisted extraction of polyphenolic compounds from black currant fruits (method of drying not given) was compared to supercritical fluid extraction, the latter being found more efficient.<sup>25</sup> Ultrasound-assisted extraction was performed in water using an ultrasonic bath of 35 kHz at different temperatures (20, 30, 40 °C) and extraction time (10, 20, 30 min). Very low content of total phenolics was found when applying ultrasound-assisted extraction, probably because water which is not highly efficient was used as extraction solvent.

An increased content by 15–25% of total phenolics from black currant fruits was found by other workers when ultrasound-assisted extraction was applied after pectinase incubation.<sup>26</sup> Ultrasound-assisted extraction was performed using an ultrasonic device of 20 kHz and 2000 W ( $60$  W  $\text{cm}^{-2}$ ) with a transducer directly dipped into the black currant juice, at amplitude 100% for 3 min 15 s.

It is worth to mention that total phenolics, total anthocyanins, ascorbic acid and total antioxidant capacity from ultrasonic extracts resulted with significant reduction in extraction times. Ultrasound-assisted extraction becomes particularly useful for rapid extraction of thermolabile compounds with any solvent system, being also much cheaper when compared to other modern techniques, such as microwave-assisted extraction. However, irrespective of the extraction technology, we found that the sample preparation highly influence the extraction yield, in particular of total anthocyanins and ascorbic acid.

## 4. Conclusions

Several ultrasound-assisted extraction parameters were optimized in order to recover the highest content of antioxidant compounds (phenolics, anthocyanins, ascorbic acid and total antioxidant capacity) in black currant fruits (frozen, freeze-dried, oven air-dried).

The optimum sonication conditions for maximum simultaneous extraction of antioxidant compounds from black currant fruits (60% ethanol with 0.15% HCl, solvent-to-sample ratio of 15/1) in relation to the sample preparation may be resumed as follow:

- (i) in all samples, the increase of the total phenolics content by ultrasonic pretreatment was not statistically significant;
- (ii) in frozen samples, the ultrasonic pretreatment proved efficiency particularly to achieve the highest content of ascorbic acid compared to conventional extraction;
- (iii) in freeze-dried samples, the ultrasonic pretreatment proved efficiency particularly to recover maximum content of total anthocyanins and total antioxidant capacity compared to conventional extraction;
- (iv) in oven air-dried samples, the ultrasonic pretreatment proved efficiency particularly to achieve high amounts of ascorbic acid and high total antioxidant activity compared to conventional extraction.

However, the highest amount of all targeted bioactives was found when freeze-drying of samples was employed, resulting in a better quality final extract. Oven air-drying at 45 °C lead to a drastic decrease of the content of ascorbic acid.

It can be concluded that ultrasound-assisted extraction might be used as a powerful tool for increasing the yield of extraction of valuable compounds from black currant fruits in strong relation to the operating conditions.

## 5. Acknowledgements

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## Povzetek

Namen raziskav je bil ovrednotiti učinkovitost ultrazvočne priprave vzorcev pri različnih amplitudah in časih ekstrakcije glede na vsebnost in antioksidacijsko kapaciteto vzorcev črnega ribeza (*Ribes nigrum*). Poleg tega je bil proučen vpliv skladiščenja oziroma sušenja vzorca. Ekstrakcija je bila izvedena v 60 % etanolu z 0.15 % HCl pri razmerju topilo-topljenec 15/1. Rezultati kažejo, da je ultrazvočna priprava vzorcev posebej uporabna za ekstrakcijo večjih količin antocianinov iz liofiliziranih vzorcev, askorbinske kisline iz zamrznjenih ter v peči sušenih vzorcev, medtem ko je celotna antioksidacijska kapaciteta visoka pri vzorcih, skladiščenih oziroma sušenih na oba načina. Ultrazvočna priprava ni pomembno vplivala na vsebnost celotnih aromatoev.

Liofilizacija je zelo zvišala vsebnost antocianinov in askorbinske kislin, medtem ko je sušenje v peči pri 45 °C močno znižalo vsebnost askorbinske kisline. Rezultati kažejo, da ultrazvočna priprava vzorcev izboljša izkoristek ekstrakcije, kar pa je zelo odvisno od procesnih pogojev.