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Communication

The effect of ultrasound treatment on the extraction of lycopene and β-carotene from cherry silverberry fruits

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Abstract: The aim of this study was to evaluate the effect of ultrasound treatment on the efficiency of carotenoid extraction from cherry silverberries. Fruits (except for the control group) were sonicated at three power levels: 200, 400 and 600 W. Fruits were extracted in 95% ethyl alcohol. After extraction, the content of β-carotene and lycopene in the extract was determined by HPLC with the UV-Vis SPD-20A detector and an acetonitrile-methanol-dichloromethane (75:25:5) as mobile phase. The dry matter content of all three sonicated samples was lower than in the control sample. Sonication at 200 W significantly ($p < 0.05$) increased β-carotene yield (by 59%) relative to the control sample. Lycopene yield also increased significantly ($p < 0.05$) relative to the control group after sonication at 200 W, but further increase in power did not induce significant improvement in extraction efficiency. The results of this study indicate that ultrasound treatment at 200 W (0.25 $W \cdot cm^{-3}$) increases the efficiency of carotenoid extraction from cherry silverberries and higher power can lead to reduction of extraction efficiency.

Keywords: cherry silverberry; ultrasound treatment; extraction; lycopene; β-carotene; carotenoids

1. Introduction

Cherry silverberry (*Elaeagnus multiflora*) fruits have potential applications in the food processing industry as a rich source of nutrients with health-promoting properties. Cherry silverberries have antioxidant, anti-inflammatory and antiproliferative properties [1,2] due to a high content of carotenoids, exogenous amino acids, macronutrients and micronutrients, unsaturated fatty acids, and vitamin C [3,4]. Fruits are a valuable source of lycopene, that seems the most potent antioxidant among common carotenoids that is renowned for its anticarcinogenic effects [4–7]. Fresh and processed silverberries have been long used in the treatment of cough, diarrhea, gastric ailments, pruritus and even cancer and bone disease [1,4]. Cherry silverberries are used in the production of various foods, including juice, herbal tea, wine, soup, sauces, desserts, candy, pudding, ice-cream topping, fruit leather, jam and jelly [6], mainly in Asia and Eastern Europe. Cherry silverberries have potential uses in the production of functional foods, tinctures, medicinal products and dietary supplements due to their high content of β-carotene and lycopene [8].

Nutrients and bioactive compounds such as β-carotene and lycopene can be isolated from cherry silverberries by extraction. Extraction is a process that involves mass transfer (and in some cases, also heat transfer), where the product has to be adequately prepared, usually ground or sliced, to increase the surface area of processed tissues and to facilitate diffusion. Additional processing methods can also be applied to increase extraction efficiency, where high power ultrasound (HPU) treatment can be one of such methods [9]. This type of treatment takes place in two steps: preliminary processing and complementary processing. During pretreatment, fruits are sonicated to degrade cell membranes and tissues (including the skin) by acoustic cavitation. Complementary processing involves sonication of the extraction and solvent mixture during the process, to induce microflows and enable the solvent to thoroughly penetrate damaged plant tissues [10,11].

The efficiency of ultrasound treatment before or during extraction has been confirmed in many fruits and vegetables [12–16], yet it has never been used to extract β-carotene and lycopene from cherry silverberries. There is also a general scarcity of research where ultrasound treatments with different power levels were applied to the same product. One of the few studies was conducted by Purohit and Gogate [14] who evaluated the efficiency of ultrasound-assisted extraction of β-carotene from waste carrot residue at different levels of ultrasonic irradiation and observed that extraction yield increased with a rise in ultrasound power. These findings indicate that acoustic cavitation induced by ultrasound of varied intensity can influence extraction yields. However, the optimal parameters of the acoustic wave, in particular its power, can differ for various products because high-power ultrasound is not a standardized technology, and it needs to be scaled up for every new application [17]. The optimal ultrasound power for extraction can also be affected by the stability of delicate compounds such as lycopene which is highly sensitive to high temperature and oxygen, and is readily converted from trans to cis isomers [18,19]. Therefore, care must be taken to minimize the loss of lycopene through oxidation or isomerization during extraction to accurately account for cause–effect changes [4]. In view of the above, the aim of this study was to evaluate the effect of ultrasound treatment at different power levels on the efficiency of lycopene and β-carotene extraction from cherry silverberry fruits.

2. Materials and methods

2.1. Samples preparation

The experiment was performed on cherry silverberry fruits of selected biotypes cultivated in the Experimental Garden of the Department of Horticulture at the University of Warmia and Mazury in Olsztyn. Fruits were collected at the optimum ripening time at the beginning of July in 2018. The harvested fruits were stored (up to 72 hours) in air-tight plastic bags at a temperature of 5 °C until analysis. The fruits selected for the experiment were fully ripe and free of damage. The pedicels were removed, the fruits were washed and divided into four groups: A—control sample; B, C, D—experimental samples for ultrasound treatment at different power levels.

2.2. Ultrasound treatment

Before extraction, cherry silverberries from groups B, C and D were subjected to ultrasound treatment in the Scientz 650 E generator (Ningbo Scientz Biotechnology Co. Ltd., China) with a titanium ultrasonic probe (7 mm tip diameter) (Ningbo Scientz Biotechnology Co. Ltd., China) emitting a sound wave with a frequency of 23 kHz. Fruit samples of 130 g each were placed in a perforated bag and immersed at the bottom of a vessel filled with water (0.8 dm^3), with the tip of the ultrasonic probe positioned around 2 cm above the sample. Every pretreatment lasted 2 minutes during which the probe was repeatedly activated and deactivated in one-second intervals to prevent probe damage and to prevent an increase in the temperature of the liquid and immersed fruits. The ultrasound treatment was conducted at three power levels: 200 W (sample B), 400 W (sample C) and 600 W (sample D), which correspond to ultrasound power density of 0.25, 0.50 and 0.75 $W \cdot cm^{-3}$, respectively, which is 0.247, 0.494 and 0.741 $W \cdot g^{-1}$ respectively. Liquid mixing and cavitation bubbles were barely discernible at 200 W, they were noticeable at 400 W, and highly noticeable at 600 W.

2.3. Extraction

Each of the four fruit samples (three ultrasonically pretreated samples and one control sample) were crushed manually without damaging the pit. Mashed samples of 50 g each were placed in 200 g of ethanol (95%) heated to a temperature of 40 °C. Despite the ethanol is not the best solvent for the extraction of carotenoids, it was chosen because it is alcohol used for preparation of tinctures valued in Central and Eastern Europe. The extraction process lasted 150 minutes in a water bath at constant temperature. The mixture was stirred every 15 minutes. After 150 minutes, the extract was removed, passed through filter paper and subjected to further analyses.

2.4. Determination of moisture and dry matter content

The moisture content of whole fruits (with the pit) was measured in all sonicated samples and in the control sample to determine whether ultrasound treatment promoted the loss of fruit juice and nutrients. Moisture content was determined with the use of a standard method [20] by drying fruit samples at a temperature of 70 °C and pressure of approximately 13.3 kPa for 6 hours. The same approach was used to measure the dry matter content of fruits after extraction and to determine whether ultrasound treatment enabled the solvent to fully penetrate plant tissues. Moisture content was expressed in grams of water per gram of dry matter, and dry matter content of moist fruits was expressed as a percentage.

2.5. Analysis of the content of bioactive compounds

The four extracts were passed through quantitative filter paper. Aliquots of 2 mL of each extract were dried under a nitrogen stream, and the resulting sediment was dissolved in 1 mL of petroleum ether. The samples were immediately assayed in the Shimadzu HPLC system (Shimadzu, Japan). Analyte absorption was measured at 450 nm wavelength with the UV-Vis SPD-20A detector (Shimadzu, Japan). Absorption peaks were read in the Chromax 2010 v. program (Pol-Lab, Poland). The peaks were separated on the Jupiter C18 column (Phenomenex, USA) with an acetonitrile-methanol-dichloromethane (75: 25: 5) mobile phase. The flow rate of the mobile phase was 1 mL/min. Sigma Lycopene (L9879) and β-Carotene (C4646) standards were used (Sigma, USA). Standard solutions were prepared by dissolving individual components in petroleum ether. The concentrations of lycopene and β-carotene in standard solutions were determined at 0.02 mg/mL and 0.01 mg/mL, respectively.

2.6. Statistical analysis

Significant differences between group means were determined at $p < 0.05$. The non-parametric Mann-Whitney U test was used due to small sample size. The analyses were carried out in Statistica 12 (StatSoft Inc., Tulsa, OK, USA). The results are presented as indices in Table 1.

3. Results and discussion

The moisture content of cherry silverberries subjected to ultrasound treatment at different power levels is presented in Table 1. The treatment exerted no significant effect on the moisture content of fresh fruits ($p > 0.05$). However, ultrasound treatment clearly influenced successive stages of extraction because the dry matter content of fruits after extraction was considerably lower in all three sonicated samples than in the control samples (Figure 1). These observations imply that acoustic cavitation induced by ultrasound induced sufficient structural damage to allow solvent penetration and greater saturation of fruit tissues with ethanol [17].

Based on the above findings, higher concentrations of bioactive compounds, such as lycopene and β-carotene, could also be expected in extracts of pretreated samples relative to the control sample due to enhanced extractability with solvents, because those carotenoids are located in cell walls and chromoplasts which both can be also the main barriers for their accessibility [21]. However, an analysis of β-carotene and lycopene concentrations (in µg per 1 gram of whole fruits subjected to extraction) in extracts of pretreated fruits, results of which are shown in Table 1, revealed that high-power ultrasound treatment significantly affected extraction yields ($p < 0.05$).

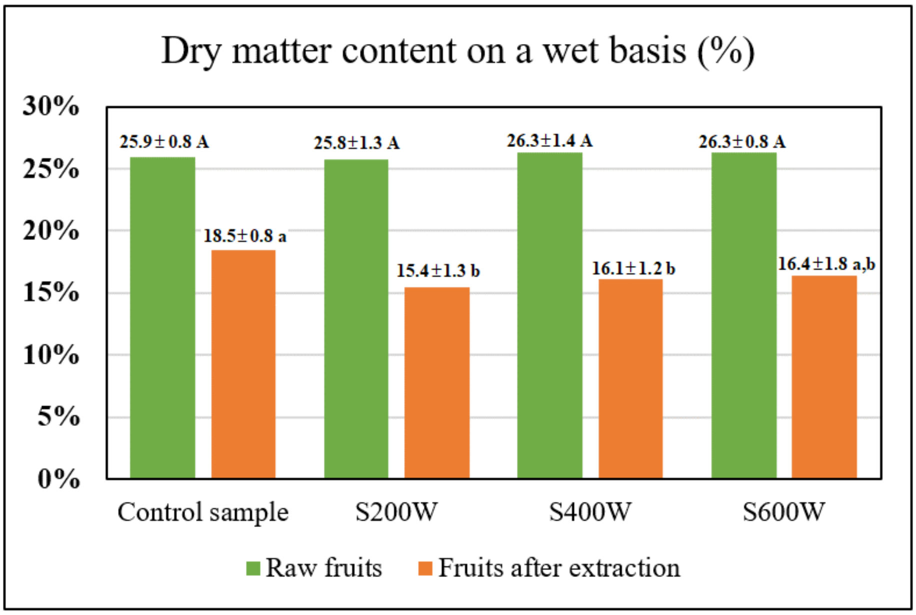

Figure 1. The effect of ultrasound treatment on the dry matter content of cherry silverberry fruits before and after extraction. Figure shows mean values with standard deviations of the mean (the results were averaged over three measurements). Identical letters (big in case of raw fruits and small in case of fruits after extraction) in different columns indicate that the mean values do not differ significantly at a confidence level of 95% ($p > 0.05$).

The same effect can be observed in literature, e.g. using similar power level of ultrasounds (200 ± 10 W) for juice from quite similar fruits (cape gooseberry, *Physalis peruviana L*) resulted significant increase of both β-carotene and lycopene concentrations [13]. But results obtained in this work showed also that ultrasound power was an important consideration. A significant increase in the extraction yields of both β-carotene (by 59%) and lycopene (by 106%) was noted only under exposure to the 200 W ultrasound treatment (0.25 $W \cdot cm^{-3}$). The application of higher ultrasound power, even during short pretreatment (2 minutes), decreased carotenoid extraction yields. In samples subjected to ultrasound power of 400 W (0.5 $W \cdot cm^{-3}$) and 600 W (0.75 $W \cdot cm^{-3}$), significant differences in lycopene extraction yield were not observed (despite a decreasing trend), whereas a clear decrease in β-carotene extraction yield was noted relative to the control sample. The decreasing trend suggests that a further increase in ultrasound power would lead to a further drop in extraction yields. The above may result from two reasons: a) the thermolabile character of the evaluated substances, b) the change of the structure of the fruit pulp. What speaks for the first explanation is that lycopene and β-carotene are highly sensitive to oxidation under exposure to high temperature and free radicals [18,19,22,23]. Sonication induces acoustic cavitation during which temperature can increase even up to 5000 °C and pressure up to 500 atm [24]. At higher ultrasound power, intensive acoustic cavitation and the accompanying hot-spots could contribute to the degradation of both lycopene and β-carotene, which both are thermolabile compounds [25]. Cavitation also stimulates the generation of hydroxyl radicals from water molecules inside fruits [26,27], and both analyzed compounds are potent antioxidants [5,6]. The production of hydroxyl radicals was probably intensified under exposure to higher ultrasound power, which could contribute to the degradation of lycopene and β-carotene. Another possible explanation of the decrease of carotenoids content in the extract with the rising ultrasound power is the change of the inner structure of the fruit pulp due to ultrasound homogenization. In the case of 200 W treatment, ultrasound could break the protein-carotenoid complexes causing the increase of carotenoids extractability [28]. Off course the treatment with higher ultrasound power could cause such effect to, but those treatment could cause also the higher homogenization of the fruit pulp and therefore formation of a stronger network entrapping carotenoids in the matrix with pectins [29], making it less accessible for extraction. However, the reason of the above can be to short time of pretreatment. The effect of longer ultrasound pretreatment on carotenoids extraction should be studied in the future.

Table 1. Average moisture content of raw and pretreated fruits and the content of bioactive compounds in cherry silverberry (Elaeagnus multiflora) extracts with mean standard errors given in brackets.

Sample	Moisture content of pretreated fruits (g_{H_2O}/g_{dm})	β-carotene content of extract (μg/g_{fm})	Lycopene content of extract (μg/g_{fm})
A: Control sample	2.861 ± 0.081[a]	1.927 ± 0.017[a]	0.520 ± 0.010[a]
B: S200W	2.884 ± 0.136[a]	3.069 ± 0.050[b]	1.071 ± 0.60[b]
C: S400W	2.812 ± 0.145[a]	1.744 ± 0.253[a]	0.681 ± 0.101[a]
D: S600W	2.797 ± 0.035[a]	1.052 ± 0.035[c]	0.463 ± 0.022[a]

Units explanation: $g_{H_2O}/g_{dm.}$–mass of water referred to dry mass of substance, μg/g_{fm}–mass of constituent referred to a mass of a fresh sample; Identical letters in the same column indicate that the mean values do not differ significantly at a confidence level of 95% ($p > 0.05$).

4. Conclusions

The results of this study indicate that only short-term ultrasound treatment with estimated power density of 0.25 W·cm^{-3} significantly ($p < 0.05$) increases the efficiency of carotenoid extraction from cherry silverberries, whereas too high power levels can lead to the degradation or decrease of accessibility of the analyzed carotenoids and can decrease extraction yields. For the extraction of β-carotene and lycopene ultrasound power of 200 W (approx. 0.25 W·cm^{-3}) seems to be optimal. Further research is required to determine the exact optimal ultrasound power density (oscillating around 0.25 W·cm^{-3}) with greater accuracy. The analyzed carotenoids are highly sensitive, therefore, efforts should also be made to optimize the duration of ultrasound treatment and extraction temperature. In the future, the extraction yields from ultrasonically pretreated fruits can also be compared with the efficiency of ultrasound-assisted extraction.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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