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Effect of storage on oxidative stability, polyphenol content and antioxidant activity of fat fraction extracted from chokeberry and blackcurrant seeds by ultrasound-assisted process

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INTRODUCTION & AIM

Pomace, the solid residue left after the extraction of juice from fruits, is a by-product generated in large quantities by the fruit industry. Traditionally, pomace has been considered waste or used in low-value applications like animal feed or composting. However, recent research has highlighted the potential of pomace as a rich source of bioactive compounds, particularly oils and other valuable components, making it a sustainable material for the extraction of these compounds.

Several extraction techniques are being researched and developed for their effectiveness in recovering oils and bioactive compounds from pomace. The most popular is conventional solvent extraction. This method may not be environmentally friendly due to the use of chemicals and their potential residues. That is why, traditional methods are being gradually replaced by alternative, like ultrasound-assisted. Ultrasonic waves help disrupt cell walls and enhance the release of compounds. This technique improves extraction efficiency, reduces time, and uses less solvent [1].

The oil obtained from chokeberry and blackcurrant seeds can be characterized by high content of bioactive compounds which naturally prevent oil from oxidation during storage. Application of ultrasound-assisted extraction can enhance their extractability.

Thus, in the following study, **the aim was to investigate the effect of storage on the oxidative stability, polyphenol content and antioxidant activity of chokeberry and blackcurrant seed oils extracted by an ultrasound-assisted process.**

METHODS

MATERIAL The research material consisted of seeds obtained from the pomace left after pressing chokeberry **(CH)** and blackcurrant **(BC)** juices. The seeds were ground using a laboratory mill. Then, 5 grams of the prepared material was weighed into Falcon tubes. The solvent used for extraction was n-hexane in S/L ratio 1/6.

Fig 1. Chokeberry and blackcurrant seeds.

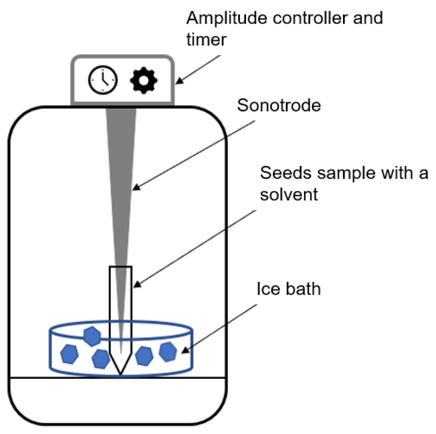

Fig 2. The scheme of ultrasonic system.

EXTRACTION The ultrasound-assisted extraction was performed using an ultrasonic processor Hielscher UP400S with a 400W power output and 24 kHz frequency. In a tube with seeds and hexane the sonotrode was immersed to the appropriate depth. The tube was placed in an ice bath and monitored with an immersion thermometer to ensure the sample temperature did not exceed 45 °C. Two variations were used: 60% ultrasound amplitude for 12 min **(US60/12)** and 90% amplitude for 6 min **(US90/6)** [2]. Conventional extraction was conducted by placing tubes with hexane in 40 °C water bath for 2h under constant agitation **(C)**.

OXIDATIVE STABILITY The oxidation induction times were measured using pressure differential scanning calorimetry (PDSC) with a Thermal Analysis DSC Q20 apparatus. The measurements were conducted under isothermal conditions at 120 °C, with a pressure of approximately 1400 kPa [3].

Fig 3. DSC Q20 apparatus.

FATTY ACID PROFILE The fatty acid profile was determined using a YL6100 gas chromatograph equipped with a flame ionization detector. Fatty acids were derivatized into methyl esters (FAME), which were identified by comparing the relative retention times of peaks with the FAME standard (Supelco 37 Component FAME Mix) [4].

Fig 4. GC YL6100 apparatus.

BIOACTIVE COMPOUNDS EXTRACTION AND DETERMINATION To obtain the polyphenol fraction from the oil, a liquid-liquid extraction was performed with hexane and methanol/water mixture. After repeating procedure 3 times, solvent mixture was evaporated to dryness under reduced pressure, and the extract was reconstituted in methanol. The extracts were then analyzed spectrophotometrically in order to determine total polyphenol content (TPC) using Folin-Ciocalteu reagent and antioxidant activity using ABTS and DPPH radicals' solutions. The absorbance results were interpreted by comparing to standard callibration curves of gallic acid and Trolox. Results were given as gallic acid equivalent per 100 g of oil (mg GAE/100 g) for TPC and as Trolox equivalent per 100 g of oil (μmol TE/100 g) for ABTS and DPPH.

STATISTICAL ANALYSIS All the studies were conducted right after the extraction and after 3 months of storage in dark, cool place. The data were statistically processed by conducting one-way ANOVA and Tukey's post hoc test. The significance level was set at p = 0.05.

RESULTS & DISCUSSION

OXIDATIVE STABILITY The oxidation induction times (OIT) shown in Table 1 indicate that before storage the ultrasound effect was only slighlty noticeable. However, after 3-month storage time oil extracted from blaccurrant seeds with the use of ultrasound (90% amplitude and 6 min time) had longer OIT than other blackcurrant oils. In terms of chokeberry oil, the effect of ultrasound treatment on OIT was not observed.

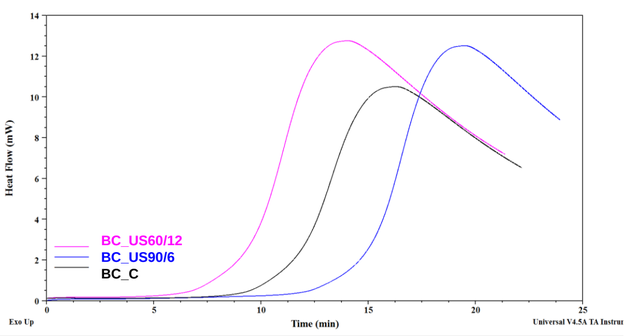

Fig 5. Example of PDSC curves of blackcurrant seed oils after 3-month storage.

Table 1. Oxidation induction time results.

Sample	Before storage	After 3-month storage
	OIT (min)	OIT (min)
CH_C	40.9 ± 0.4^{e}	38.4 ± 0.5^{E}
CH_US60/12	41.0 ± 0.1^{e}	31.6 ± 0.6^{C}
CH_US90/6	40.0 ± 0.3^{d}	34.7 ± 0.3^{D}
BC_C	22.8 ± 0.3^{c}	11.6 ± 0.3^{B}
BC_US60/12	20.6 ± 0.3^{b}	9.4 ± 0.2^{A}
BC_US90/6	18.9 ± 0.2^{a}	13.7 ± 1.7^{B}

FATTY ACID PROFILE The dominant fatty acid in chokeberry and blackcurrant seed oils was linoleic fatty acid with percentage shares around 70% and 45%, respectively. It was observed that fatty acid profile was not dependent significantly on the extraction method. That is in agreement with previous findings which stated that extraction method did not influence chemical composition of oil [5]. Also, fatty acid profile remained stable during storage.

TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY The extraction method positively influenced the polyphenol content in blackcurrant seed oil (Fig 7) and the antioxidant activity against ABTS radicals (Fig 6) in both oils. The smallest decrease in TPC after storage occurred in blackcurrant seed oil extracted using sonication at 60% amplitude in 12 min. Similarly, the lowest reduction in antioxidant activity against ABTS radicals after storage was observed in samples extracted with ultrasound (60% amplitude, 12 min). In contrast, for antioxidant activity against DPPH radicals (Fig 8), the lowest reduction was seen in samples obtained through classical extraction. Increased antioxidant activity in oils extracted applying ultrasound was previously observed [6].

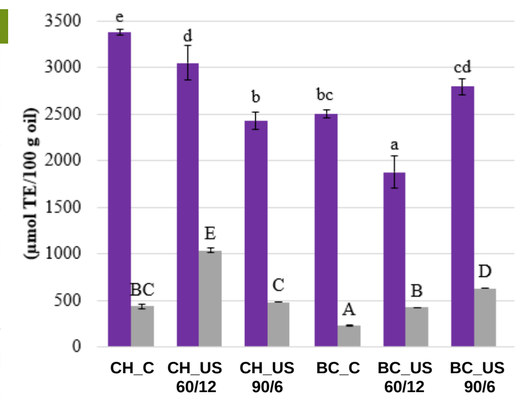

Fig 6. Antioxidant activity against ABTS in fresh oils (purple color) and after 3-month storage (grey color).

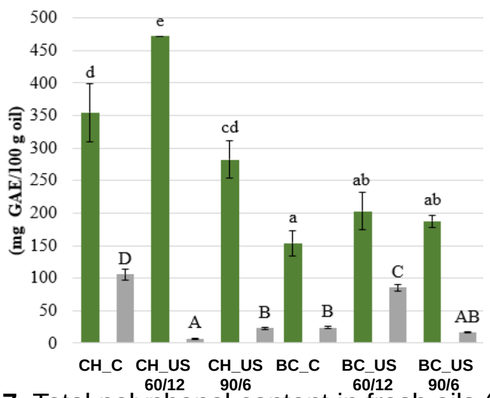

Fig 7. Total polyphenol content in fresh oils (green color) and after 3-month storage (grey color).

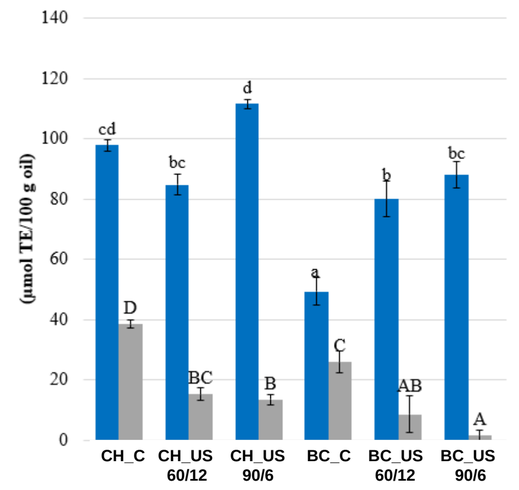

Fig 8. Antioxidant activity against DPPH in fresh oils (blue color) and after 3-month storage (grey color).

CONCLUSION

The use of sonication with appropriately selected amplitude and time parameters in the extraction process was beneficial, as it shortened the extraction time and improved the oxidative stability of the chokeberry and blackcurrant seed oils. Also, applying ultrasound during extraction may improve extractability of bioactive compounds and improve natural antioxidant properties of bioactive compounds abundant in oils. Further analysis of bioactive compounds profile and their stability during storage is needed.

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