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Liberation and recovery of phenolic antioxidants and lipids in chokeberry (*Aronia melanocarpa*) pomace by solid-state bioprocessing using *Aspergillus niger* and *Rhizopus oligosporus* strains

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ABSTRACT

The present study focused on the changes in polyphenols, antioxidant activities and lipid compositions from chokeberry pomace during solid-state fermentation (SSF) with *Aspergillus niger* and *Rhizopus oligosporus*. The extractable phenolics increased more than 1.7- fold during both fermentation processes. A similar trend was observed for total flavonoids. The obtained results also indicated that a longer fermentation period resulted in a greater loss of anthocyanins. The free radical scavenging ability of phenolic extracts, evaluated by DPPH and TEAC assays, were significantly enhanced during the SSFs. HPLC-MS analysis of phenolic compounds showed that the amounts of flavonols and cinnamic acids increased while the concentrations of glycosylated anthocyanins decreased substantially. The SSF of chokeberry pomace also resulted in a significant increase in lipids with high linoleic acid content (57–61% of the total fatty acids). The present investigation demonstrated that SSF enriched the chokeberry pomace with phenolic antioxidants and lipids with better nutritional-quality characteristics.

1. Introduction

Due to the growing interest in healthy foods, the popularity and demand for chokeberries (*Aronia melanocarpa*) is increasing in the United States and worldwide. The chokeberries having a significantly higher polyphenolic content and antioxidant activity then other berries (Zheng & Wang, 2003), in recent years have started to be cultivated on large scale by more and more farmers from Eastern Europe (Sójka, Kołodziejczyk, & Milala, 2013). The fruits are processed mainly into juice (over 90% of the production), thereby producing significant quantities of by-products, characterized by high content of dietary fibres (63–78% in dry matter (DM) of pomace), flavanols, proanthocyanidins with high degree of polymerizations (88.2–90.5% of total polyphenolics), and anthocyanins (7.7–9.3% of total polyphenolics) (Sójka et al., 2013). Chokeberry pomaces (especially the seed-rich pomace) contain also saccharides (2.7–3.5% in DM of pomace), proteins (18–24% in DM) and lipids (9.8–13.9% in DM) with high levels of unsaturated fatty acids (Dulf, Andrei, Bunea, & Socaciu, 2012; Sójka et al., 2013). Due to this composition, the chokeberry pomaces are prone to rapid microbial growth. Albeit the potential of chokeberry by-products as sources of health-promoting hydro- and lipophilic phytochemicals is well documented, there is lack of information in scientific literature on efficient and economical solutions for the liberation and recovery of these biologically active molecules from the pomaces.

The biopretreatment of agro-food wastes by using filamentous fungi in solid-state fermentation (SSF) system is an efficient, cost-effective and environmentally friendly path to improve the recovery rates of valuable biomolecules (Madrera, Bedriñana, & Valles, 2017; Ni et al., 2015).

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To the best of our knowledge, no study has investigated the effects of SSF with filamentous fungi on polyphenolics, antioxidant activity and total lipids of chokeberry by-products. Therefore, the purpose of this research was to study the influence of solid-state fermentation with *Aspergillus niger* and *Rhizopus oligosporus* (generally recognized as safe (GRAS)) on the content of phenolic compounds, antioxidant activities and lipid compositions in chokeberry (*Aronia melanocarpa*) pomaces.

2. Material and methods

2.1. Materials and chemicals

The chokeberry (cultivar "Nero") pomace was obtained from a local juice production company (Romania) and was dried in oven at the temperature below 40 °C, and ground to particle-size fractions of 0.5–1 mm and stored at a temperature of −18 °C before use. All chemicals and solvents used were of analytical grade.

2.2. Microorganisms and culture conditions

The fungal strains *Aspergillus niger* (ATCC-6275) and *Rhizopus oligosporus* (ATCC-22959) (LGC Standards GmbH, Wesel Germany) were maintained on potato dextrose agar (PDA) at 4 °C. Inoculating cultures were produced by growing the strains on fresh PDA at 27 °C for 10 days before use. The spore inoculum was prepared by washing the agar surface with sterile distilled water.

2.3. SSF

Chokeberry pomace was homogenized and moisturized (65%) with a nutrient solution containing (g/l): yeast extract, 1 and glucose, 30. The pH of the pomace was adjusted to 5.5 with saturated KOH solution. After, 40 g aliquots of the homogenate were transferred into 500 mL Erlenmeyer flasks, which were then autoclaved at 121 °C for 30 min and inoculated with spore suspension (2×10^7 spores/g of solid). The flasks were incubated under static conditions at 30 °C during 12 days and samples were withdrawn at different time intervals and stored at −20 °C. The experiments were conducted in triplicate.

2.4. Polyphenolic composition

Phenolic compounds from pomaces (1 g) were extracted with 20 mL of extraction mixture (hydrochloric acid: methanol: water in the ratio 1: 80: 19) in an ultrasonic bath for 30 min at 40 °C (Dulf, Vodnar, Dulf, & Toşa, 2015). The mixtures were centrifuged at 4000 g for 10 min and the supernatants were filtered under vacuum through the glass frit. The filtrates were evaporated to dryness under vacuum. The resulting extracts were stored in methanol (4 °C) until analysis (total phenolics, flavonoids and anthocyanins; individual phenolics; antioxidant activities).

The total phenolic content was estimated by the Folin-Ciocalteu method and was expressed as gallic acid equivalents (GAE) in mg/100 g dry weight (DW) of pomace (Dulf, Vodnar, & Socaciu, 2016).

The total flavonoid level (expressed as quercetin equivalent (QE) in mg/100 g DW of pomace) was determined by the aluminum chloride colorimetric method as described previously (Dulf et al., 2016).

Total monomeric anthocyanins, expressed as mg of cyanidin-3-*O*-glucoside (Cy3G) per 100 g DW of pomace were quantified by pH-differential method according to Giusti and Wrolstad (2001).

Individual polyphenolic analyses were conducted on an Agilent 1200 HPLC with DAD detector, coupled with MS detector single quadrupole (Dulf et al., 2016). Methods of quantification and identification of cinnamic acids (expressed in mg chlorogenic acid/100 g DW of pomace), flavonols (mg rutin/100 g DW of pomace) and anthocyanins (mg cyanidin/100 g DW of pomace) have been described in our previous paper (Dulf et al., 2016).

2.5. Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of the non- and fermented chokeberry pomace extracts was evaluated spectrophotometrically by a slightly modified method of Brand-Williams, Cuvelier, and Berset (1995) as described by Dulf et al. (2015).

The percentage inhibition (I%) was calculated using the following formula:

I (%) = [1− (test sample absorbance/blank sample absorbance)] x 100.

Trolox equivalent antioxidant capacity (TEAC) assay was carried out as described by Bunea et al. (2011). The results were expressed as micromol Trolox equivalents per gram sample (μmol TE/g DW of pomace).

2.6. Lipid extraction and fatty acid analysis

The pomace samples (5 g) were extracted with 60 mL chloroform:methanol (2:1, v/v) (Dulf et al., 2016). Total lipid (TL) contents were determined gravimetrically. An aliquot (10–15 mg) of each TL extract was transesterified intofatty acid methyl esters (FAMEs) using the acid-catalyzed procedure (Dulf, Oroian, Vodnar, Socaciu, & Pintea, 2013) and analyzed by gas chromatography–mass spectrometry (GC-MS) using a previously described method (Dulf et al., 2016). Quantification of the fatty acids was achieved by the comparison of peak areas with internal standard (nonadecanoic acid, Sigma, Steinheim, Germany) which was added to the samples (200 μg) prior to methylation, without application of any correction factor. Fatty acid compositions of TLs in chokeberry pomaces were expressed as molar percentages of the fatty acids.

2.7. Statistics

All measurements were performed in triplicate and the results were presented as mean ± standard deviation (SD). Correlations among the different antioxidant activity assays and phenolics were calculated using Pearson's correlation. Statistical analyses were performed by Student's *t*-test and ANOVA (repeated measures ANOVA; Tukey's Multiple Comparison Test; GraphPad Prism Version 5.0, Graph Pad Software Inc., San Diego, CA). The values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Total phenolics, flavonoids and anthocyanins contents

The total phenolic (TP) content of fermented chokeberry pomaces was estimated using the Folin-Ciocalteu method (Fig. 1A). The measurable phenolics in both SSF process increased significantly ($p < 0.05$) during the growth period. In the case of fermentation with *R. oligosporus* there was a gradual increase until day 2 after which the TP content rapidly increased more than 1.8- fold from an initial value of 950.96 mg GAE/100 g DW of pomace to 1778.70 mg GAE/100 g DW of pomace (day 6), before decreasing sharply and then stabilizing by day 12. For the SSF with *A. niger* the evolution of phenolic content follows a typical sigmoid growth curve, the TP level gradually increasing until day 9 (more than 1.7- fold, to the maximum value of 1703.50 mg GAE/100 g DW of pomace) before saturating (Fig. 1A). Correia, McCue, Magalhães, Macêdo, and Shetty