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How solid-state fermentation with *A. niger* changes agro-waste of apple, aronia and sea buckthorn to attractive antioxidant compounds

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Abstract: This work focuses on the influence of solid-state fermentation on the polyphenols and flavonoids conent in fruit pomace of apple, aronia, and sea buckthorn. A comparison was made during a seven-day fermentation of pomace with *A. niger* and with autochthonous microflora, showing that this method has no significance for aronia and sea buckthorn pomace. Autochthonous microflora had a more favorable effect on the content of polyphenols and flavonoids than *A. niger*. However, in the case of apple pomace, the content of total polyphenols increased up to 1425 mg GAE/100 g DW on the seventh day of fermentation with *A. niger*, representing a 7.3-fold increase compared to the initial value of 195 mg GAE/100 g DW. Our results therefore present an attractive way of agro-waste utilization and the subsequent implementation of these isolates in other types of industry.

Keywords: fruit pomace, solid state fermentation, total phenolic content, total flavonoid content, *Aspergillus niger*

Introduction

Waste from fruit and vegetable processing industry has great potential as a source of specific bioactive compounds ranging from proteins to essential fatty acids and polyphenol (Banerjee et al., 2017; Kumar et al., 2017). In Europe, juice production residue ranks as the 5th main contributor to the total yearly food waste (Roda-Serrat et al., 2021). This waste is nowadays used mainly as livestock feed, soil fertilizer, raw material for biofuel production, or it is discarded in landfills. Recovery of bioactive compounds from food processing waste therefore is an attractive opportunity that has received a lot of attention in recent years (Madeddu et al., 2021; Majerska et al., 2019).

Apples are one of the most favorite fruits, mainly due to their delicious taste, high nutritional value, and good storability, depending on the variety. They are also known for their high antioxidant content (Musacchi and Serra, 2018). Chemical composition of apples may vary depending on the variety, production area, harvesting, and storage procedures. In general, an apple contains more than 80 % of water, carbohydrates (fructose, glucose, sucrose), organic acids, vitamins (mainly vitamin C), minerals, and fiber.

Berries, especially black chokeberry (aronia), black elderberry, black currant, blueberry, sea buckthorn, and others are also an excellent source of a wide range of antioxidant substances. The wide range of phytochemicals present in the above-mentioned plants, as well as their different concentration profiles, provide different health benefits in each genus and species (Lavefve et al., 2020). Berries are rich in minerals, vitamins, fiber, and phenolic compounds with high biological activity. High levels of vitamin C, carotenoids, and phenolic compounds in berries give them high antioxidant potential, which is why berries are considered the most important source of antioxidant molecules in human diet.

Antioxidant activity is caused by compounds with protective activity against oxidation. Although these can be found in various fruits, their bioavailability for human organism may be limited as most of these substances are found in conjugated form, where sugar residues are attached to hydroxyl groups. Studies show that these compounds can be released or converted into more active forms by fermentation. They attribute these changes to metabolic activities affecting phenolic acids, flavonoids, tannins, release of antioxidant peptides, changes in vitamin content, and production of exopolysaccharides. For example, filamentous fungi can produce β-glucosidase, which releases polyphenols bound to cell wall structures and increases the extractable content of polyphenols (Verni et al., 2019; Waldbauer et al., 2017).

Fermentation is an important part of food biotechnology. Initially, it was used to preserve food products and prevent diseases. The process has evolved with human civilization to the extent that production and extraction of bioactive compounds is possible (Martins et al., 2011). The two types of fermentation commonly used in the industry are Submerged Fermentation (SmF) and Solid-State

Fermentation (SSF). The principle of SmF consists in a liquid medium as a source of nutrition for microorganisms so that fermentation can take place. SSF is the same process but in the absence or near absence of free water in the medium. The substrate itself is required to contain the necessary moisture for the microorganism to survive and grow (Erskine et al., 2023). SSF is a more suitable candidate for commercial enzyme production compared to SmF due to the higher yield achieved using the same strain. Although not as conventional as SmF, SSF has attracted interest from both the commercial and the scientific worlds in recent years. As it is solid-state fermentation, it has significantly lower water and energy requirements, which in turn reduces bacterial contamination along with sterilization and production costs (Hansen et al., 2015).

Aspergillus niger is a microscopic filamentous fungus growing on organic matter in the presence of oxygen. It is widely distributed in nature and is found in soil, decomposing plant material, or compost. The conditions for its growth include a wide range of pH values, from 1.4 to 9.8 with the optimum between 4–6.5, and temperature range of 6–47 °C with optimal temperature between 24–37 °C. This versatility, together with the water activity of 0.88–0.98 required for optimal growth and sporulation, makes it an industrially attractive organism with simple cultivation. It is used in the food industry, specifically for food enzymes production. *A. niger* fermentation is dependent on substrates containing sucrose, which increases the production costs; however, a cheaper and more sustainable substrate can be used (Passamani et al., 2014; Upton et al., 2017).

The goal of our work was to use agro-waste of apple, aronia, and sea buckthorn processing focusing on fermentation with *A. niger*, which offers efficient transformation of waste into a source of valuable antioxidants that can be further used in the food, pharmaceutical, or cosmetic industry.

Materials and methods

Chemicals

Aronia pomace was prepared from fruits of "Leikora" cultivar, pressed at ecofarm Aronia Agro s.r.o Žiar nad Hronom SR). Sea buckthorn pomace was from "Nero" cultivar berries, obtained from Tvrdošovce agricultural cooperative (poľnohospodárske družstvo Tvrdošovce, SR). Apple pomace was prepared from "Golden Delicious" cultivar, pressed at McCarter, a.s. (Dunajská streda, SR).

Aspergillus niger (CCM 8189) was grown on selective YGC agar (yeast extract glucose chromamphenicol agar) (Biolife, Italia) and Tween 80 (Merck Life Science, SR) was used as surface-active substance to create spore suspension. Composition of the mineral nutrient medium for solid state fermentation was prepared with chemicals from Centralchem (SR) (Sodium nitrate p.a., Potassium dihydrogen phosphate p.a., Potassium chloride p.a., Magnesium sulfate heptahydrate p.a., Copper sulfate pentahydrate p.a.) and Lachema Brno (CR) (Zinc sulfate hydrate p.a., Ferrous sulfate monohydrate p.a.).

Other chemicals used in the analytic methods were purchased from VWR (CR) (HPLC water and HPLC methanol), Centralchem (SR) (Ethanol 96 %, Glacial acetic acid, Folin-Ciocalteu reagent p.a., Sodium carbonate anhydrous p.a.), Merck Life Science (SR) (Gallic acid of analytical purity, Quercetin ≥ 95 %, Eriodictyol ≥ 98 %).

Preparation of spore suspension

A. niger CCM 8189 was borrowed from the Institute of Biochemistry and Microbiology (originally purchased from the Masaryk University in Brno). It was inoculated on selective YGC agar (yeast extract glucose chromamphenicol agar) (Biolife, Italia) and grown at 30 °C for 5–7 days. After incubation, sterile 0.1 % Tween 80 solution was used to loosen the spores from the surface of the agar medium. The spore suspension was poured through sterile gauze into a sterile, flat-bottomed, wide-necked titration flask using a funnel. The number of spores/ml was determined by the cultivation method.

Solid state fermentation (SSF)

In each substrate, the number of colony forming units was first determined by the cultivation method. Their numbers were $9.1 \cdot 10^1$ CFU/g for apple pomace, $9.3 \cdot 10^1$ CFU/g for sea buckthorn pomace, and $9.8 \cdot 10^1$ CFU/g for aronia pomace. The substrate suitable for SSF was prepared according to Gulsunoglu et al. (2020), with 5 g of dried and ground fruit pomace weighted and 25 ml of distilled water added, and enriched with the minerals listed in Table 1. Next, 1 ml of the spore suspension ($4.8 \cdot 10^6$ spores/ml) was added to

Tab. 1. Composition of the mineral medium (Gulsunoglu et al., 2020).

Compound	Content [g/l]
$NaNO_3$	6.00
KH_2PO_4	1.52
KCl	0.50
$MgSO_4 \cdot 7H_2O$	0.50
$ZnSO_4 \cdot H_2O$	0.01
$CuSO_4 \cdot 5H_2O$	0.01
$FeSO_4 \cdot H_2O$	0.01

the Erlenmeyer flasks with the weighed substrate, which was then closed with a stopper. Samples fermented with autochthonous microflora consisted of substrate enriched only with the mineral medium. All Erlenmeyer flasks were incubated for 7 days at 30 °C in aerobic conditions. Samples were taken on the 0th, 3rd, and 7th day always from three parallels.

Sample preparation and extraction

Preparation and extraction of dried fermented samples were carried out as follows: 2 g of each sample were weighed; extraction solvent was prepared by mixing water and food-grade ethanol in a 20:80 ratio (H_2O:EtOH) and 20 ml of the prepared solvent were added to each weighed sample. Extraction was performed in an ultrasound bath for 30 minutes. Once the extraction was complete, the extracts were filtered to remove solids, and the filtrate was evaporated under pressure below 18 mbar at 30 °C until the solvent was removed. The prepared extracts were stored in a freezer at –30 °C.

Determination of total polyphenol content and other reducing compounds

Total content of polyphenols (TPC) and other reducing compounds, including certain amino acids, proteins, and other antioxidants, in fermented fruit pomace was determined using a modified method (Zhu et al., 2023) with gallic acid as the standard and the Folin-Ciocalteu reagent. Concentration of phenolic compounds and other reducing compounds was determined based on the calibration curve prepared by analyzing the individual dilutions of the gallic acid standard with gallic acid concentration ranging from 0,0001 to 1 mg/ml. The prepared concentrations were dosed as 100 µl into test tubes and 500 µl of Folin-Ciocalteu's reagent was added. After 3 minutes, 1500 µl of 20 % sodium carbonate solution was added. Finally, everything was topped with 7900 µl of distilled water. The same preparation was used for the samples, except for them being diluted in the ratio of 10:1. A blank was prepared in two parallels containing distilled water instead of the sample. Finally, the entire rack was wrapped in aluminum foil and the solutions were let to react in the dark for 2 hours at room temperature. After two hours, the samples were vortexed and pipetted (250 µl) onto a 96-well microplate from a BioTek Reader spectrophotometer. Absorbance was measured at the wavelength of 765 nm using the BioTek Reader spectrophotometer. All samples, calibration solutions and blanks were dosed onto the plate in two parallel measurements. TPC values were determined as the equivalent of gallic acid in milligrams per 100 g of dry matter (mg GAE/100 g DW).

Determination of total flavonoid content

Total flavonoid content (TFC) in the samples was determined spectrophotometrically using a solution of aluminum chloride and quercetin as a standard (Kreft, 2002). First, calibration solutions were prepared by gradually diluting a quercetin stock solution to concentrations of 0.001, 0.002, 0.003, 0.01, 0.02, and 0.03 mg/ml. Then, 2 ml of each calibration solution was placed into a test tube and 0.2 ml of a 5 % aluminum chloride solution was added. The fermented samples were diluted in UV ethanol and prepared analogically to the calibration solutions, 2 ml of the sample was pipetted into the test tube and 0.2 ml of a 5 % aluminum chloride solution was added. For the blank, the preparation was identical, only instead of the sample, UV ethanol was used. Each sample was vortexed thoroughly and allowed to react at room temperature for 30 minutes. Then, the samples were pipetted onto the plate of a BioTek Reader spectrophotometer in 250 µl portions for analysis. A parallel sample was also pipetted for each sample, calibration solution and blank. Absorbance was measured at the wavelength of 420 nm. Total flavonoid content (TFC) was evaluated as quercetin equivalent in milligrams per 100 g of dry matter (mg QE/100g DW).

Determination of specific flavonoids

Reversed-phase high-performance liquid chromatography was used to determine eriodictyol and quercetin employing two methods (Gulsunoglu et al., 2020), (Liu et al., 2021) which were modified several times to achieve the best possible separation. Modifications comprised varying both stationary and mobile phases, mobile phase gradient, injection volume, and concentration. Finally, conditions listed in Table 2 were applied.

Tab. 2. Conditions used in specific flavonoids analysis.

Column	SunShell C18, 2.6 µm, 4.6 × 150 mm
Mobile phase	A — water with the addition of 0.1 % acetic acid, B — methanol
pH of phase A	2.8
Gradient for phase B	0 — 15 min 20 — 50 %, 15 — 20 min 50 — 70 %, 20 — 40 min 70 %
Flow rate	1.000 ml/min
Injection	5 µl
Pressure	204 bars
Temperature	30 °C
Detector	MWD
Wavelength	280 nm
Length of analysis	40 minutes

Evaporated extracts were used for the analysis; 10 mg of each extract were weighed and dissolved in 1 ml of HPLC methanol. Subsequently, the sample was filtered through a 0.22 μm microfilter into vials. Content of the given polyphenol in the sample was determined using a calibration curve considering quantitative parameters, specifically the peak area. First, a mixed standard was prepared, knowing the elution times of both standards. In the work, the standard for eriodictyol and quercetin were used. Stock solution was prepared in the ratio of 1:1 and HPLC grade methanol was used as a solvent. Next, calibration solutions were prepared in concentrations of 0.05, 0.04, 0.03, 0.02, 0.01, 0.008, 0.005, and 0.003 mg/ml, and the calibration curve was expressed as the dependence of the peak area on the concentration of the mixed standard. This calibration curve was then used to calculate the concentrations of the specific polyphenols in the fermented samples.

Statistical analysis

All data obtained experimentally were processed by descriptive statistics in the Microsoft Office program – Excel 365. When determining the total content of phenolic compounds and total flavonoid compounds, the values were reported as the average of three individual experiments and the error bars were expressed as confidence interval. When determining the content of specific polyphenols, the values were reported as the average of three measurements ± standard deviations.

Results and discussion

Total polyphenol content (TPC) and other reducing compounds in apple pomace

Effect of apple pomace fermentation on TPC values and other reducing compounds was more significant than that of the below mentioned berries. TPC increased with each passing day (Fig. 1). On day 0, when the substrate was freshly inoculated, the same results were recorded for fermentation with autochthonous microflora and fermentation with *A. niger* (NM). The initial value was 195 mg GAE/100 g DW; Gulsunoglu et al. (2020) stated an initial value of 350 mg GAE/100 g.

The difference between inoculated and non-inoculated substrate was established on the 3rd day when *A. niger* hydrolyzed polyphenols with the help of the produced enzymes, increasing the content of polyphenols to 939 mg GAE/100 g of dry matter, which represents an almost 5-fold increase compared to the initial value. Gulsunoglu et al. (2020) recorded only a 2-fold increase. This difference may be caused by different variety or different content of peels, stalks, cores, and pulp in apple pomace. Also, a different collection strain of *A. niger* was used. Increase of TPC values was also seen in samples fermented with autochthonous microflora. The highest content of phenolic compounds, as expected, was on the 7th day, with 1471 mg GAE/100 g of dry matter, which is 1.5 times more than on the 3rd day. Fermentation with *A. niger* provided a result 4.1 times higher than that with autochthonous

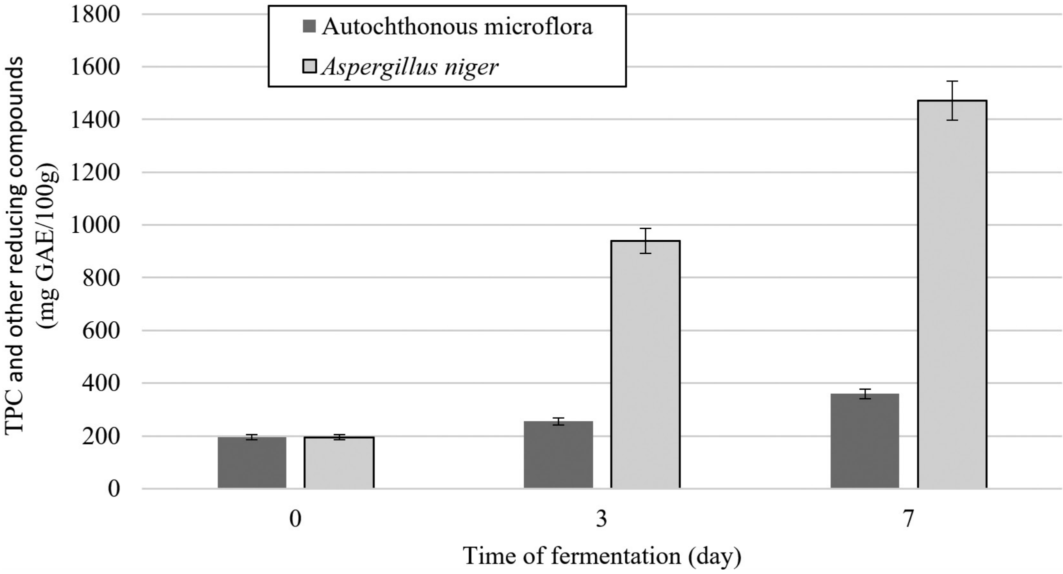


Fig. 1. Total content of polyphenolic (TPC) and other reducing compounds in apple pomace fermented with autochthonous microflora and *A. niger*.

microflora. The results are in agreement with the study by Gulsunoglu et al. (2020), which shows total content of phenolic compounds obtained by fermentation with *A. niger* of 1425 mg GAE/100 g of dry matter with slower onset but a continuous addition of phenolic compounds. The results were thus equal on the 7th day.

Total flavonoids content (TFC) in apple pomace

In non-fermented apple pomace, the total flavonoid content (TFC) typically ranges between 85 to 140 mg QE/100 g DW (Rana et al., 2015; Gulsunoglu et al., 2020). Our baseline 110 mg QE/100 g DW, aligns well with these values, and is similar to 99 mg QE/ 100g DW reported by Rana et al. (2015). Our results for TFC (Fig. 2) correspond well with findings reported in Rana et al., (2015) and Gulsunoglu et al. (2020).

Gulsunoglu et al. (2020) observed a TFC of 382 ±18 mg QE/100 g DW in apple peels fermented with *A. niger*, which is comparable to our value of 360 mg QE/100 g DW on day 7. TFC measured on day 3 of fermentation with *A. niger* (228 mg QE/100 g DW) matched the values reported by Gulsunoglu et al. (2020) well, despite our initial TFC being approximately 20 % lower. Autochthonous microflora showed only a transient increase on day 3 (150 mg QE/100 g DW), followed by a significant decline to values even lower than the initial baseline (100 mg QE/100 g DW). Similarly, the significant decline in TFC observed in the sample fermented with autochthonous microflora on day 7 parallels the degradation patterns described in their work.

The marked TFC increase to 360 mg QE/100 g DW in the *A. niger* fermented sample on day 7 correlates well with our HPLC results, which identified quercetin (20 mg/100 g) and eriodictyol (300 mg/100 g) as the predominant flavonoids present. This suggests that these two compounds are likely the major contributors to the total flavonoid content in fermented apple pomace.

Determination of specific flavonoids in apple pomace

Apple pomace contains several phenolic compounds that occur in free forms or conjugated with sugars, acids, and other biomolecules soluble or insoluble in water (De Araújo et al., 2021). However, after fermentation, there is a change in the phenolic profile of apple pomace and a change in the content. Quercetin occurs in apple pomace in various structures, when various glycosides are bound to it with a glycosidic bond, e.g. quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, and others (Gumul et al., 2021). During fermentation with *A. niger,* enzymes are produced, such as glucosidase, which cleave glycosidic bonds creating new interesting phenolic compounds. Such a compound is eriodictyol, which is mainly found in citrus fruits, but it is formed during the fermentation of apple pomace via the transformation of other flavonoids. This phenomenon has been proven by HPLC analysis (Fig. 3a, b), confirming the presence of eriodictyol in 7-day fermented apple pomace. The obtained data were compared with those of Gulsunoglu et al. (2020) in table no. 3, showing the quercetin content decrease, i.e. consumption, during fermentation. When measuring the content of eriodictyol, responses on the 0th day were not measured. However, some values obtained on the 3rd and 7th day of fermentation with autochthonous microflora were

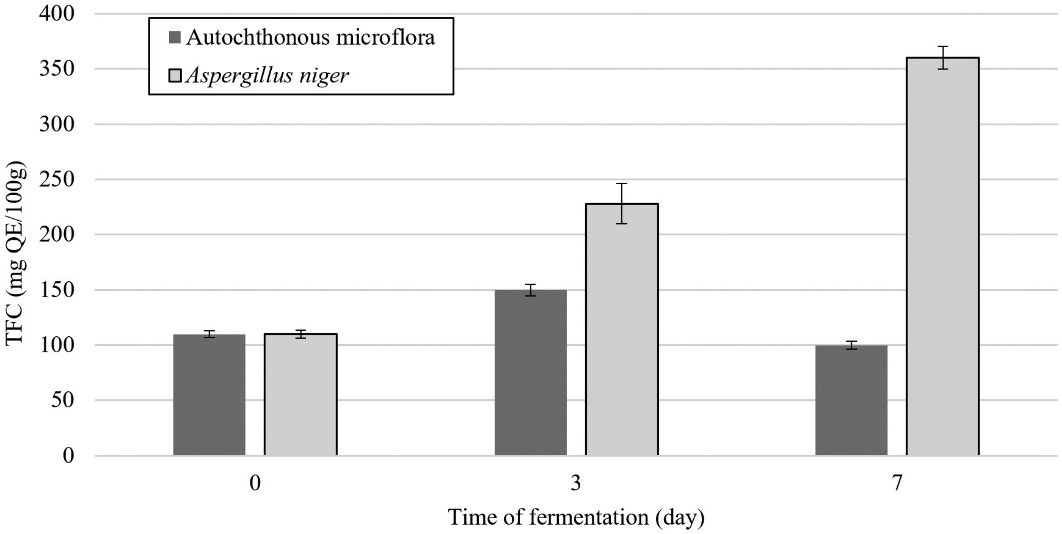
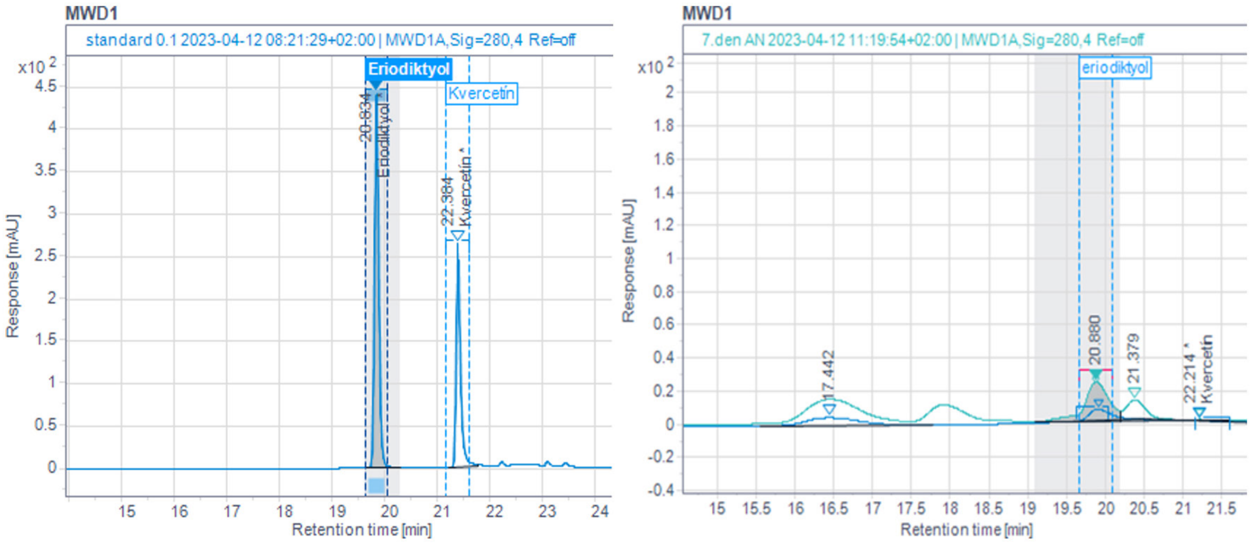


Fig. 2. Total content of flavonoids (TFC) in apple pomace fermented with autochthonous microflora and *A. niger*.



a) b)

Fig. 3. a) – HPLC analysis of 1 mg/ml of eriodictyol and quercetin in a mixture. b) – Overlap of the standards with a sample of apple pomace fermented for 7 days by *A. niger*.

Tab. 3. Effect of fermentation on the content of quercetin and eriodictyol in fermented and unfermented apple pomace.

		Results (mg/100 g DW)		**Gulsunoglu et al., (2020) (mg/100 g DW)**	
Analytes	Day	Autochthonous microflora	*A. niger* (CCM 8189)	Autochthonous microflora	*A. niger* (ZDM2)
Quercetin	0.	20.0 ±0.3	20.0 ±0.3	19.9 ±0.9	19.9 ±0.9
	3.	17.5 ±0.2	21.5 ±0.3	17.9 ±2.4	22.5 ±0.7
	7.	16.3 ±0.6	20.0 ±1.0	14.2 ±3.6	18.1 ±3.9
Eriodictyol	0.	–	–	–	–
	3.	49.2 ±0.2	203.1 ±7.9	ND	264.1 ±5.0
	7.	54.4 ±0.2	300.0 ±16.4	ND	639.2 ±14.5

not reported in the above publication (Gulsunoglu et al., 2020), probably because eriodictyol standard was not used to seek the peak overlap, which was identified by HPLC-MS/MS and HPLC-PDA.

In apple pomace fermented by *A. niger*, an increase in eriodictyol was observed. However, compared to the given publication, the increase was lower as our results were half of those presented on the 7th day of fermentation. This difference arose from a modification of their procedure, as a different drying method, different collection strain of *A. niger*, different extraction solvent, and different variety of apple pomace were used. Either of the collection strains of *A. niger* used in fermentation and subsequent extraction of phenolic compounds produces ochratoxin A. However, they belong to the 2nd risk group, which means that they can be the cause of human or animal diseases (Varga et al., 2007). The collection strain used in this paper is only suitable for laboratory research purposes, and thus for our model experiment. Our results can serve as a template for the use of the collection strain *A. niger*, used in the food and pharmaceutical industry, for example in the production of citric acid.

Levels of quercetin and eriodictyol measured in fermented apple pomace in this study are consistent with data reported in previous research. Quercetin concentrations in both fermented samples ranged between 20–21.5 mg/100 g DW, aligning closely with findings of Gulsunoglu et al. (2020), who identified quercetin as a dominant flavonoid in apple by-products, showing minimal fluctuation after solid-state fermentation (SSF) with *Aspergillus niger* (ZDM2).

Eriodictyol levels significantly increased during the fermentation process, with the highest concentration (300 mg/100g DW on day 7) recorded in samples fermented with *A. niger*. This value remains below the maximum of 639 mg/100g DW observed by Gulsunoglu et al. (2020) for a different *A. niger* strain (ZDM2).

Nevertheless, our findings support the hypothesis that *A. niger* fermentation promotes enzymatic transformation of phenolic precursors to bioactive flavonoids, with eriodictyol emerging as a key marker of fermentative bioconversion.

Total polyphenol content (TPC) and other reducing compounds in aronia samples

The total content of compounds with redox activity was determined as a TPC value of 1691 mg GAE/100 g DW in aronia pomace on the 0th day of fermentation (Fig. 4). On the 3rd day of fermentation, the values increased rapidly, reaching a value of 7880 mg GAE/100 g DW in the fermentation with autochthonous microflora, which is approximately 4.6-fold the original TPC value. In samples fermented with *A. niger*, the TPC values were 6925 mg GAE/100 g DW on the 3rd day, which is about 4-fold the original value.

Various studies on the fermentation of agro-waste to obtain biologically significant substances monitored changes in the content of polyphenolic compounds during solid state fermentation of fruit pomace. For example, Dulf et al. (2018) observed the effect of solid-state fermentation on aronia pomace using the Folin-Ciocalteu reagent method to determine TPC. Their research showed that TPC and other reducing compounds in SSF processes increase significantly during the growth period. For SSF with *Aspergillus niger*, their content of TPC gradually increased until the 9th day, when the maximum value of 1703.5 mg GAE/100 g DW represented more than 1.7-fold the initial value. In the following days of fermentation, a decrease in TPC was observed. Compared to the study by Dulf et al. (2018), higher levels of TPC and other reducing compounds were measured on each day of aronia pomace fermentation. This fact can be attributed to the different composition of the pomace, i.e. the content of skins, seeds, pulp or stems. The content of antioxidant substances can also be due to the difference in variety, growing conditions, growing place, etc. It is also necessary to consider that a different strain of microflora can cause differences in the results.

On the 7th day of fermentation, the difference between the microflora in the aronia samples was observed. While with the autochthonous microflora the values continued to increase up to the value of 11420 mg GAE/100 g DW, a trend of lower growth of TPC values was observed in the fermentation with *A. niger*. TPC values of the autochthonous microflora on the 7th day of fermentation were 1.5-fold higher compared to those of *A. niger*. The fact that the autochthonous microflora provided better results in increasing polyphenolic compounds during fermentation can be justified by the ability of natural microflora to adapt to the environment, its diversity and a wide range of enzymes, in contrast to the artificially added *A. niger*.

Total flavonoid content (TFC) in aronia samples

The total flavonoid content of aronia samples was 418 mg QE/100 g DW on the 0th day of fermentation (Fig. 5). On the 3rd day of fermentation, their content increased in both microflorae, increasing 2.3-fold, up to 952 mg QE/100 g DW, in samples fermented with autochthonous microflora, and approximately 2-fold, up to 835 mg QE/100 g DW, in samples fermented with *A. niger*. On the 7th day of fermentation, a slight increase was observed in *A. niger* samples, up to 861 mg QE/100 g DW, while in the case of autochthonous microflora,

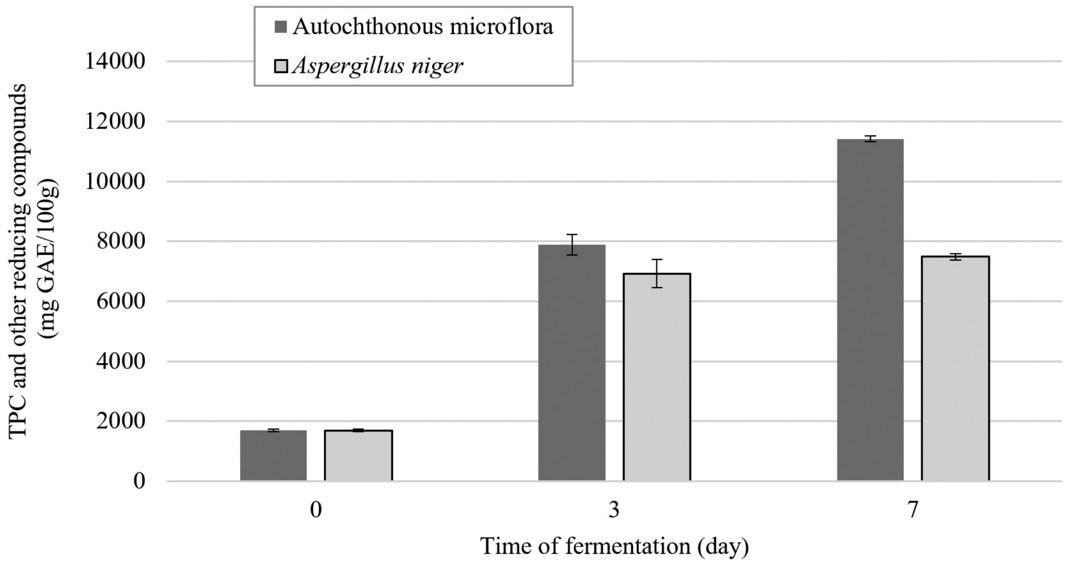


Fig. 4. Total content of polyphenolic (TPC) and other reducing compounds in aronia pomace fermented with autochthonous microflora and *A. niger*.

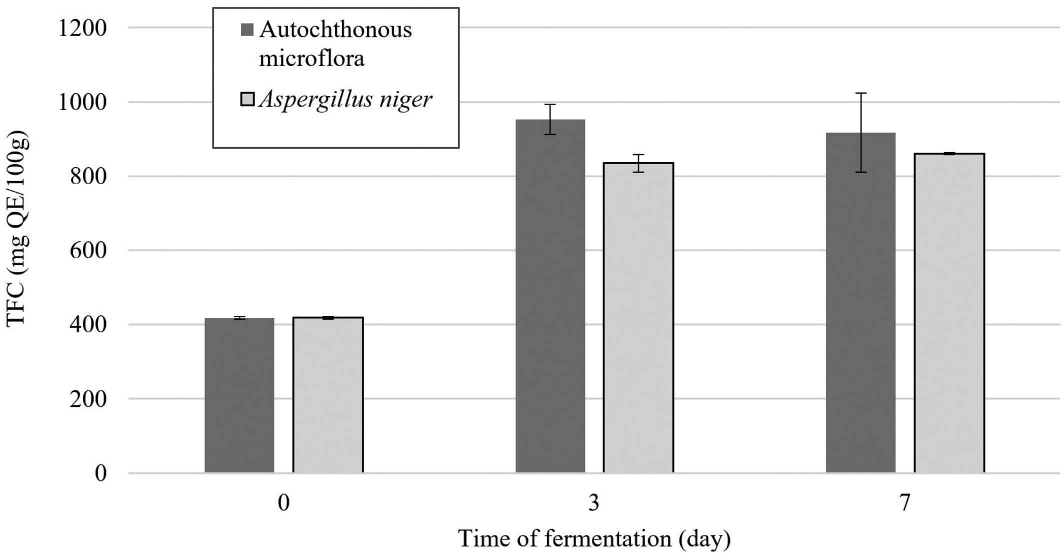


Fig. 5. Total content of flavonoids (TFC) in aronia pomace fermented with autochthonous microflora and *A. niger*.

Tab. 4. Effect of fermentation on the content of quercetin and eriodictyol in fermented and unfermented aronia pomace.

		Results (mg/100 g DW)	
Analytes	Day	Autochthonous microflora	*A. niger* (CCM 8189)
Quercetin	0	226.5 ±3.5	226.5 ±3.5
	3	207.2 ±4.1	239.8 ±5.0
	7	191.4 ±4.6	228.3 ±3.9
Eriodictyol	0	ND	ND
	3	59.4 ±2.6	182.7 ±3.9
	7	66.5 ±2.1	269.4 ±5.5

on average, the values decreased down to 917 mg QE/100 g DW.

Results for aronia samples can be compared with those of Dulf et al. (2018), in which the total amount of flavonoids in fermented aronia pomace showed similar trends as total polyphenols. When *A. niger* was used for solid-state fermentation, the TFC level in their study increased 1.5-fold by day 9 (to 264.00 mg QE/100 g DW) compared to the initial value (176.00 mg QE/100 g DW) followed by a decline during the remaining growth period. The increase in total flavonoid content was greater in SSF with *R. oligosporus,* increasing 1.6-fold by day 6 (to 277.00 mg QE/100 g DW) before gradually decreasing during the remaining fermentation period. This course of fermentation is analogic to our measurements with aronia pomace, where the content of total flavonoids differs numerically, which may be caused by the aspects mentioned above, but their content increases with the course of fermentation. Aronia pomace fermented with *A. niger* showed similar increase in our (2-fold) and in the comparative (1.5-fold) study. An analogic course of the effect of fermentation on TFC (increase in values) was observed also in a study with plum pomace (Dulf et al., 2016). However, again, we can state that the naturally present microflora had a more efficient composition of microorganisms considering that it had a better effect on increasing TFC during the fermentation of aronia pomace. Therefore, it can be stated that there is potential to follow up on our study with fermentation experiments of aronia pomace using other microbiological strains to find the ones more effective in increasing TFC values.

Determination of specific flavonoids in aronia pomace

Quantification of quercetin and eriodictyol in aronia pomace during solid-state fermentation (Tab. 4.) revealed notable dynamics in flavonoid composition. The initial quercetin concentration of 226.5 ±3.5 mg/100 g DW in non-fermented pomace aligns with values reported in literature. Sosnowska et al. (2022) observed total quercetin derivatives ranging from 199.5 to 623.6 mg/100 g DW depending on the ripening stage and cultivar. Our data fall into the lower–intermediate range, likely reflecting varietal, climatic, and processing differences.

Throughout fermentation, quercetin levels remained relatively stable in samples inoculated with *Aspergillus niger*, with a slight increase by day 3 and stabilization at 228.3 ±3.9 mg/100 g DW on day 7. In contrast, a gradual decrease was observed in samples fermented with autochthonous microflora. This suggests that *A. niger* promotes limited hydrolysis of glycosidic forms into free quercetin without significant degradation.

Eriodictyol, undetectable in unfermented pomace, emerged as a key flavonoid during fermentation. Its concentration increased to 182.7 ±3.9 mg/100 g DW by day 3 and peaked at 269.4 ±5.5 mg/100 g DW on day 7 in *A. niger*-fermented samples, while its values remained significantly lower in the autochthonous treatment. Formation of eriodictyol can be attributed to the enzymatic activity of *A. niger*. These findings are consistent with observations by Dulf et al. (2018), who reported enhanced flavonoid bioavailability after fermenting aronia pomace with *A. niger*.

Total polyphenol content (TPC) and other reducing compounds in sea buckthorn pomace

Using the Folin-Ciocalteu reagent method to determine TPC and other reducing compounds resulted in a value of 1324 mg GAE/100 g DW in sea buckthorn pomace on the 0th day of fermentation (Fig. 6). On the 3rd day, TPC values increased to 1943 mg GAE/100g DW in the autochthonous microflora samples (>1.4-fold) and to 1976 mg GAE/100 g (approximately 1.5-fold) in *A. niger* samples. On the 7th day of fermentation, a slight decrease was observed in both microflora samples, 1773 mg GAE/100 g DW for the autochthonous microflora and 1648 mg GAE/100 g DW for *A. niger* (Fig. 6). A similar experiment was performed by Dulf et al. (2015), where two Sambucus species were fermented with *Aspergillus niger*. On the 3rd day of fermentation, the maximum increase in TPC values was observed, the values decreased and stabilized in the following days. In their study, this fermentation progress was explained by the lack of nutrients, so a limited amount of certain nutrients for optimal growth and enzymatic processes can be assumed also in sea buckthorn samples.

Total flavonoids content (TFC) in sea buckthorn pomace

TFC values in sea buckthorn samples showed different trend to aronia samples (Fig. 7). On the 0th day of fermentation, TFC of 518 mg QE/100 g DW was measured and the content of flavonoids decreased during the fermentation in the presence of both microflorae. On the 3rd day of fermentation, only 0.4-fold of the original flavonoid content was measured in autochthonous microflora samples, while it was 0.6-fold of the initial value, decreased to 307 mg QE/100 g DW, in *A. niger* samples.

The 7th day of fermentation showed even lower values. During the fermentation with autochthonous microflora, TFC values decreased down to 184 mg QE/100 g DW (35.5 % of the initial content of total flavonoids). In samples fermented with *A. niger*, TFC of only 235 mg QE/100 g DW (approximately 45 % of the content from day 0) were determined. A possible explanation is that SSF with *Aspergillus niger* increases the total polyphenol content in sea buckthorn pomace by releasing bound phenolics and transforming flavonoids into simpler phenolics. At the same time, the total flavonoid content probably decreases due to enzymatic degradation,

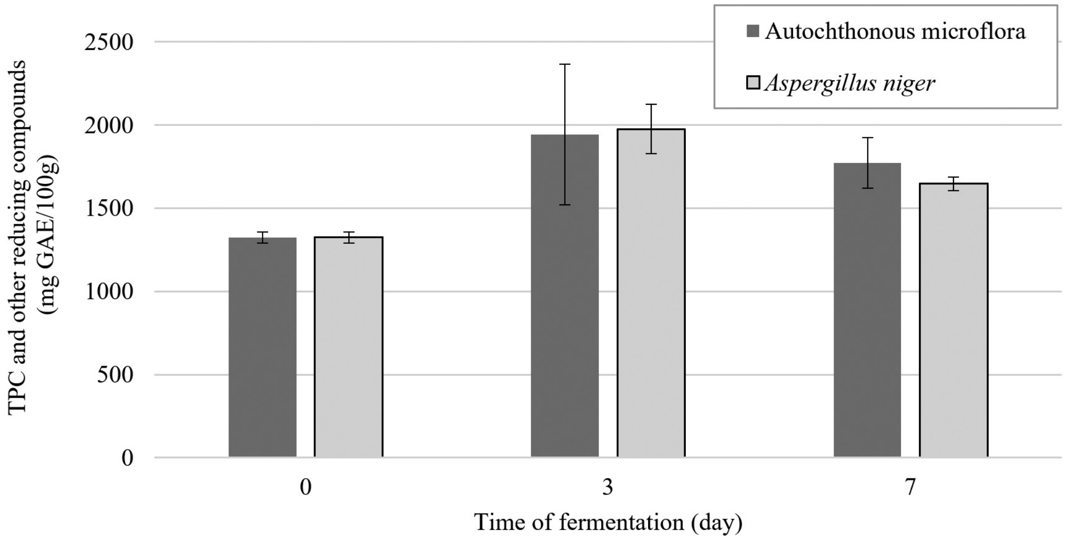


Fig. 6. Total content of polyphenols (TPC) and other reducing compounds in sea buckthorn pomace fermented with autochthonous microflora and *A. niger*.

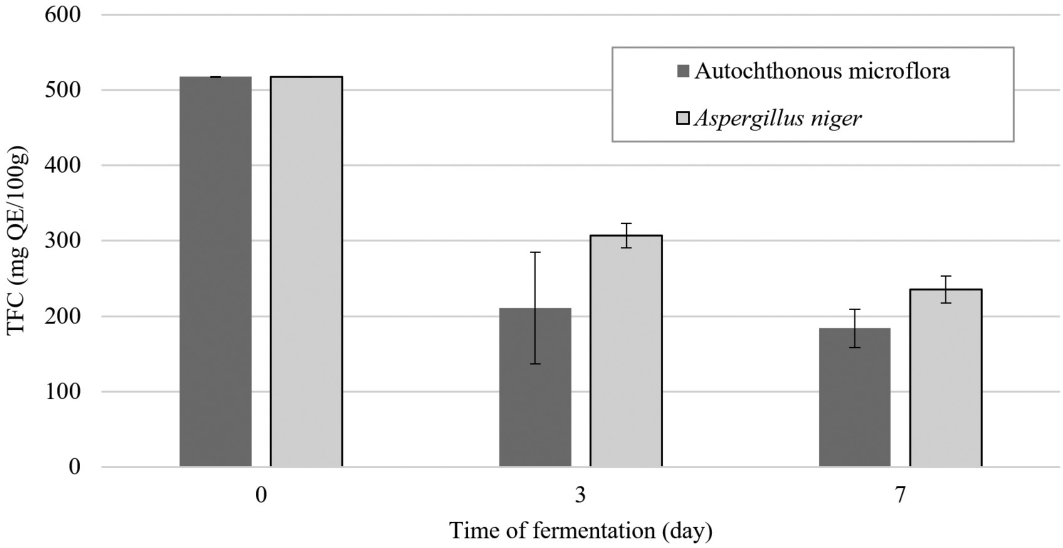


Fig. 7. Total content of flavonoids (TFC) in sea buckthorn pomace fermented with autochthonous microflora and *A. niger*.

Tab. 5. Effect of fermentation on the content of quercetin and eriodictyol in fermented and unfermented sea buckthorn pomace.

		Results (mg/100 g DW)	
Analytes	Day	Autochthonous microflora	*A. niger* (CCM 8189)
Quercetin	0	9.1 ±0.3	9.1 ±0.3
	3	7.6 ±0.4	8.5 ±0.3
	7	7.2 ±0.3	7.9 ±0.2
Eriodictyol	0	ND	ND
	3	27.3 ±1.5	95.6 ±2.3
	7	35.4 ±1.9	146.8 ±3.1

oxidation, or fungal metabolism. There are currently no published studies on the influence of fermentation on the total flavonoid content in sea buckthorn pomace, to compare our results.

Determination of specific flavonoids in sea buckthorn pomace

Initial quercetin content in sea buckthorn pomace was 9.1 ±0.3 mg/100 g DW (Tab. 5), closely matching the values reported by Dienaitė et al. (2020), who found 7.6–9.0 mg/100 g DW in sea buckthorn pomace fractions. Fermentation with either autochthonous microflora or *A. niger* led to a modest reduction in quercetin concentration by day 7. In samples fermented with autochthonous microflora, the quercetin content decreased to 7.2 ±0.3 mg/100 g DW, while in those fermented with *A. niger*, it reached 7.9 ±0.2 mg/100 g DW. This reduction suggests either partial degradation of quercetin or its transformation into secondary metabolites during the fermentation process.

During fermentation, both *A. niger* and autochthonous microflora produce various enzymes, including β-glucosidase, peroxidases, and phenoloxidases, which can hydrolyze glycosidic bonds (e.g., quercetin-3-O-glucoside to aglycone quercetin) and subsequently oxidize or catabolize aglycones. Oxidative enzymes may further convert quercetin into quinone structures or open the flavonoid ring, leading to the formation of phenolic acids or other low-molecular-weight metabolites (Hur et al., 2014).

Eriodictyol was not detectable in the initial material but was observed as early as the 3rd day of fermentation. In samples fermented with *A. niger*, the concentration increased significantly up to 146.8 ±3.1 mg/100 g DW on day 7. A similar, though less pronounced, increase occurred in autochthonous fermentations (35.4 ±1.9 mg/100 g DW). These results confirm the role of fungal fermentation in phenolic profile modification, particularly in matrices such as sea buckthorn, which are naturally rich in glycosylated flavonoids. The observed eriodictyol levels suggest partial enzymatic conversion from precursor compounds during solid-state fermentation.

To date, no peer-reviewed studies have explicitly reported the fermentation of sea buckthorn pomace with *Aspergillus niger*. Most available publications focus on either the fermentation of sea buckthorn juice or the application of lactic acid bacteria. Although sea buckthorn pomace contains valuable flavonoids, its fermentation with filamentous fungi remains underexplored. The absence of direct studies in this context indicates a promising opportunity for future research into fungal valorization of sea buckthorn by-products.

Conclusions

This study demonstrated that solid-state fermentation (SSF) using *Aspergillus niger* significantly influences the polyphenolic profile of fruit pomace substrates, albeit in a substrate-specific manner. In apple pomace, fermentation with *A. niger* resulted in a 7.3-fold increase in total polyphenol content (TPC) and more than a 3-fold increase in total flavonoid content (TFC), with quercetin and eriodictyol identified as major contributors. These findings support the potential of fungal SSF as an efficient valorization strategy for apple by-products.

In contrast, the effects of fermentation on aronia and sea buckthorn pomace were less pronounced. While both types of pomace showed an increase in TPC during early fermentation stages, the results revealed superior performance of autochthonous microflora compared to *A. niger*, particularly in terms of sustained phenolic enrichment. However, in aronia pomace, *A. niger* induced a significant increase in eriodictyol content, reaching 269.4 mg/100 g DW by day 7, while maintaining quercetin levels. This bioconversion aligns with literature describing fungal enzymatic release and transformation of glycosylated flavonoids.

In sea buckthorn pomace, both TPC and TFC values decreased after day 3 of fermentation despite an increase in eriodictyol concentration, particularly in *A. niger*-fermented samples. This suggests that although fungal fermentation enhances specific bioactive compounds, it may also lead to the degradation of flavonoid structures in substrates with limited nutrient availability.

Overall, the findings underscore the promising potential of SSF with *A. niger* in enhancing the content of bioactive compounds in apple pomace. In case of aronia and sea buckthorn, future studies should investigate optimized fermentation parameters and explore alternative microbial strains to achieve improved flavonoid bioconversion.

Acknowledgements

This publication was supported by the Slovak Scientific Grant Agency VEGA based on contract no. 1/0141/23.

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