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AKADÉMIAI KIADÓ

Enrichment of apple juice with antioxidant-rich elderberry (*Sambucus nigra* L) pomace extract

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ABSTRACT

Elderberry pomace, a by-product generated by elderberry processing industries, may be a favourable resource for further utilisation due to its components of high nutritional quality. In our research, elderberry pomace extract (EP) was added to apple juice as natural food additive for controlling microbial spoilage and enriching antioxidant components.

During the 8-week storage period of enriched apple juice (EPA) and control apple juice samples antioxidant properties were evaluated using the FRAP assay, Folin–Ciocalteu method, and pH differentiation method. The amount of polyphenols components was quantified using an RP-HPLC method. The microbiological status of samples was studied by determining the total viable and yeast/mould counts. The EPA is an important source of polyphenol components and other bioactive compounds, and the results suggest that extract of elderberry pomace could be a promising natural preservative to improve microbiological stability during refrigerated storage and increase the quality of apple juice.

KEYWORDS

elderberry, pomace, antioxidant, polyphenol, viable count, yeast/mould

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1. INTRODUCTION

Wild and cultivated species of elderberry (*Sambucus nigra* L.) have been used in folk medicine for thousands of years to prevent countless diseases (Charlebois, 2007). The medicinal potential of elder is due to its large number of bioactive compounds, polyphenols, phenolic acids, flavonoids, catechins, proanthocyanidins, which help the human body's defence against accumulated free radicals (Sidor and Gramza-Michałowska, 2015; Koss-Mikołajczyk et al., 2016).

The main step in the production of elderberry juice is usually pressing, during which the liquid phase is separated from the solid phase. The by-product of pressing is 25–40% of the mass of all berries, which contains parts of the skin and seeds of the fruit. Further utilisation of fruit pomace is recommended due to its valuable and biologically active compounds (Zeng et al., 2023). The EP has a large amount of significant polyphenols and anthocyanins, reaching up to 75–98% of the polyphenol and anthocyanin content of the whole berry. According to Radványi et al. (2013), a polyphenol content of 200–1,100 mg/100 g and an anthocyanin content of 600–1,600 mg/100 g can be measured in EP, which are close to the results measured in elderberries.

In addition, there is a negative acceptance of using synthetic additives worldwide, so the interest in researching preservatives of natural origin is gaining more and more ground (Tarnavölgyi, 2008; Rout et al., 2022). There is a growing interest in food components with a potential health-protecting capacity. Biologically active, antimicrobial, and bioregulatory substances found in fruits are often the focus of research. The fruits contain bioactive secondary plant substances, like polyphenols, phenolic acids, flavonoids. These classes of substances provide defence mechanisms for plants against microorganisms, insects, and plant pests (Sun et al., 2002).

Therefore, in our research, we enriched apple juice (EPA) by adding an elderberry pomace extract rich in antioxidants, and changes in antioxidant content and microbial status were monitored during 8 weeks of storage.

2. MATERIALS AND METHODS

2.1. Materials

The elderberry (*S. nigra* L.) was of the 'Haschberg' variety from the BOTÉSZ ('Bodzatermelők Értékesítési Szövetkezete') plantation in Nagyvenyim (46° 57′ 28″ N; 18° 51′ 53″ E). The EP was produced based on the industrial practice.

The chemicals used during the analytical measurements came from Reanal Laboratory Chemicals Ltd., while the materials required for microbiological measurements were provided by Merck Life Science Ltd.

2.2. Sample preparation

The EP was dried at atmospheric pressure in a LP-322 type oven at 60 °C to a moisture content of 5% (m/m), then, after shredding, the pomace powder was extracted with 20% (v/v) acetone for 1 h using ultrasound bath (35 kHz). After filtration, the solvent was evaporated by drying at atmospheric pressure in an oven at 60 °C.

After dilution with water, the elderberry pomace extracts were used in apple juice production. The 70 °Brix dry matter apple concentrate (provided by Sió-Eckes Ltd.) was diluted to reach the typical dry matter value of 100% apple juice (11.2 °Brix) (Control) with two different concentrations of EP (in accordance with the FVM decree 152/2009, XI. 12) (EPA-1, EPA-2). Table 1 contains the composition of the apple juices produced in the experiment.

After mixing, the apple juices were filled into bottles, heated to 80 °C using a water bath and heat treated for 15 min. After a rapid cooling, they were stored at 10 °C for 8 weeks.

2.3. Analytical methods

The samples were stored for 8 weeks and were sampled every two weeks. The values of TPC, FRAP, and TAC were examined for all samples (Hitachi U-2900 spectrophotometer). The amounts of the main polyphenolic compounds of the initial and final samples were determined by RP-HPLC measurement. Total viable cell count and total yeast/mould count were determined. The experiment was carried out with 3 parallel measurements.

2.3.1. Determination of total polyphenol content (TPC). The total polyphenol content (TPC) was measured based on the method of Singleton and Rossi (1965) (λ = 760 nm). Results are given in gallic acid equivalents (mg GAE/L).

2.3.2. Determination of antioxidant capacity (FRAP). The antioxidant capacity (Ferric Reducing Ability Power) was measured based on the method of Benzie and Strain (1966) (λ = 593 nm). Results are expressed in ascorbic acid equivalents (mg AAE/L).

2.3.3. Determination of total monomeric anthocyanin content (TAC). The total anthocyanin content was determined using the pH differentiation method of Lee and Finn (2007) (λ = 520 and 700 nm). Results are expressed in cyanidin 3-glucoside equivalents (mg CGE/L).

2.3.4. Determination of polyphenol components. Quantification of polyphenolic components was performed using a high-performance liquid chromatography device (Shimadzu Corporation) and a Phenomenex (C18) 4.6 × 150 mm, 3 µm column (Phenomenex, Torrance, California, USA). LC Solution Software was used to process and analyse the data.

High purity water containing 1% (v/v) formic acid (eluent A) and acetonitrile containing 1% (v/v) formic acid (eluent B) were used as solvents for gradient elution at a flow rate of 1.5 mL min^{-1} as shown in Table 2.

In all cases, detection was carried out at 280 and 310 nm. The identification of components based on the retention time of the standard molecules (catechin: 15.654 min, chlorogenic acid:

Table 1. The composition of the produced apple juices

Sample name	Apple concentrate (mL/100 mL apple juice)	Water (mL/100 mL apple juice)	EP (mL/100 mL apple juice)	Pomace concentration (mg mL^{-1})
Control	15	85	0	0
EPA-1	15	42.5	42.5	15
EPA-2	15	0	85	30

EPA: enriched apple juice; EP: elderberry pomace extract.

Table 2. Gradient elution used to separate polyphenolic molecules

Time (min)	A%	B%	Flow rate (mL min^{-1})
0	95	5	1.5
3	95	5	1.5
10	75	25	1.5
30	100	0	1.5
35	100	0	1.5
35.5	95	5	1.5
45	95	5	1.5

A: 1% (v/v) formic acid; B: acetonitrile containing 1% (v/v) formic acid.

15.953 min, epicatechin: 16.752 min, rutin: 18.282 min, quercetin-3-glucoside: 18.842 min). For quantitative analysis, the concentrations of polyphenolic compounds were determined using external four points calibration. Accordingly, the polyphenol concentrations were given in units of mg L^{-1} apple juice.

2.3.5. Determination total viable count. The determination of the total viable count was carried out by conventional plating technique with an incubation time of 72 h at 30 ± 1 °C, according to the standard MSZ EN ISO 4833-1:2014 from the initial and last samples of the storage experiment.

2.3.6. Determination yeast/mould count. The count of viable moulds and yeasts was determined in accordance with the standard MSZ ISO 21527-1:2013 using the horizontal method by culturing on a selective medium (malt agar) and incubating at 25 °C.

2.4. Statistical evaluation

SPSS Statistica V20 program was used for statistical data analysis. Differences between samples were evaluated by Student *t*-test at the 0.05 confidence level. To establish the relationships between the results, the Pearson's correlation was used at a 95% confidence level.

3. RESULTS AND DISCUSSION

According to our results, the TPC parameters of EPA-1 and EPA-2 samples were higher than of the control apple juices (Fig. 1).

The total polyphenol content of EPA-1 increased during storage until the 4th week, then followed a stagnant trend, and finally a small decrease was detected by the 8th week, so overall the initial value did not differ significantly from the value at the end of the 8 weeks of storage. The polyphenol content of EPA-2 did not change significantly during storage.

The measured FRAP results (Fig. 2) also confirmed that elderberry pomace extract significantly increases the amount of antioxidant compounds in apple juice samples. The EPA samples had significantly higher FRAP values than control apple juice.

The FRAP values were found to be reduced in all samples, in case of the EPA-2 there was a 21.4% decrease by the end of the storage time compared to the initial samples.

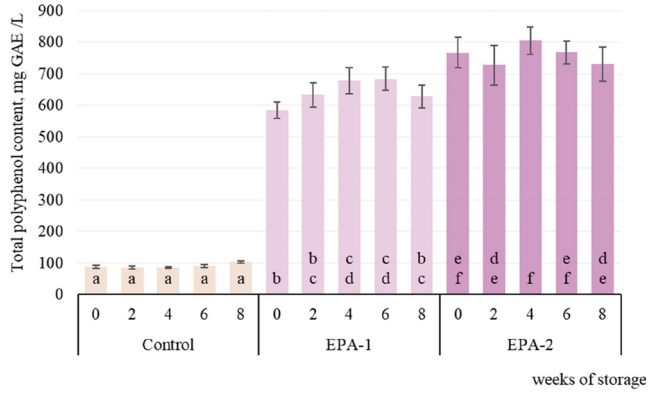

Fig. 1. Changes in total polyphenol content of apple juices during storage
Different letters in the columns indicate significant differences between the values.
EPA: enriched apple juice

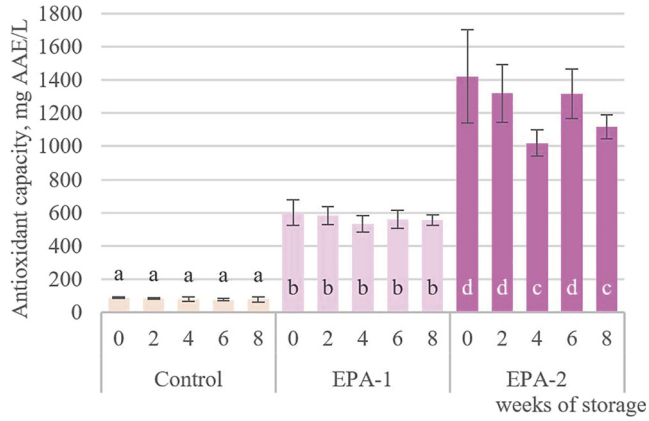

Fig. 2. Changes in antioxidant capacity of apple juices during storage
Different letters in the columns indicate significant differences between the values.
EPA: enriched apple juice

Rentsendavaa et al. (2021) reported similar results, a slight degradation of FRAP and TPC during the storage of sea buckthorn juice containing dried sea buckthorn pomace.

The measurement results of the anthocyanin content (Fig. 3) also show that enrichment of apple juices with valuable components was very successful. The TAC was found to be higher in the EPA samples in comparison with the control apple juice. The Student's *t* test for total anthocyanin content showed significant differences between all samples. The initial TAC content of the EPA-1 sample was 6,847 mg L^{-1}, and the anthocyanin content of the EPA-2 sample was 13,327 mg L^{-1}.

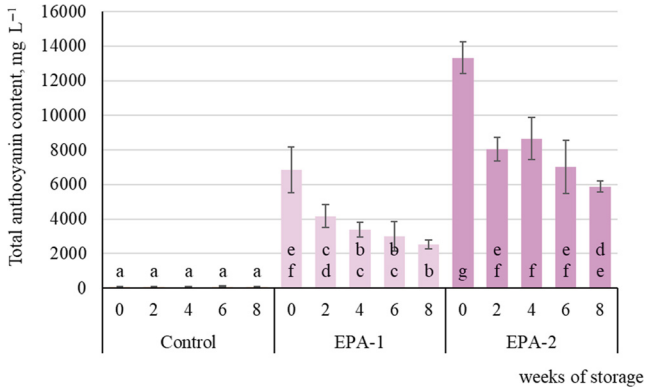

Fig. 3. Changes in total anthocyanin content of apple juices during storage
Different letters in the columns indicate significant differences between the values.
EPA: enriched apple juice

During the storage, average TAC values of the investigated apple juices samples decreased, significant differences ($P < 0.01$) were found among the initial and last samples of EPA-1 and EPA-2 for anthocyanin content, but still a considerable anthocyanin content remained in the enriched apple juice samples by the end of the storage period.

The antioxidant results obtained for control juice agree with the results of other researchers (Kahle et al., 2005; Ribárszki and Stéger-Máté, 2022). Results of EPA samples agree with the results measured in elderberry juice by Vujanović et al. (2020).

Anthocyanins appeared to be the predominant polyphenol constituents. Parameters TPC and TAC are highly related ($r = 0.82$). Phenolic compounds, including anthocyanins, exhibit strong antioxidant activity to contribute significantly to FRAP values of samples. Our results showed high correlations among values of TPC and FRAP ($r = 0.88$).

In all cases, larger amounts of identified polyphenolic components (Table 3) were found in the EPA samples than in the control juice samples, mainly amounts of catechin, epicatechin, and rutin increased significantly. The amount of these components closely correlated with the

Table 3. Polyphenolic components of apple juice samples

Sample	Storage time (week)	Catechin (mg/100 mL)	Chlorogenic acid (mg/100 mL)	Epicatechin (mg/100 mL)	Rutin (mg/100 mL)	Quercetin-3-glycoside (mg/100 mL)
Control	0	7.8 d	0.2 a	2.4 c	0.8 a	1.2 ab
	8	7.7 d	0.3 a	2.4 c	0.8 a	1.1 ab
EPA-1	0	75.3 f	0.7 a	10.1 d	8.2 d	0.9 ab
	8	62.7 f	0.0 ab	8.3 d	7.8 d	0.8 ab
EPA-2	0	126.5 g	1.9 bc	10.7 d	18.1 e	1.6 b
	8	111.5 g	0.9 a	7.5 d	19.7 e	1.9 bc

Different letters in the columns indicate significant differences between the values. EPA: enriched apple juice.

Table 4. Results of total viable and yeast/mould counts of apple juices enriched with elderberry pomace

Samples	Total viable count		Yeast/mould count	
	Initial	8th week	Initial	8th week
	(CFU/100 μL)		(CFU/100 μL)	
Control	0	48	0	6
EPA-1	2	30	0	0
EPA-2	2	2	0	0

EPA: enriched apple juice.

amount of the pomace extract. During storage, the amounts of polyphenolic components were mostly stable, but no chlorogenic acid could be detected at the end of the storage period in EPA-1. Neves et al. (2021) reported on the stability of rutin component and the degradation of the chlorogenic acid component during storage. Domínguez et al. (2020) reported a value of 0.8 mg/100 g for the rutin content of elderberries, and according to Senica et al. (2016), elderberry juice contained 23 mg/100 g epicatechin, 15 mg/100 g catechin, 6.3 mg/100 g chlorogenic acid, 1.7 mg/100 g quercetin-3-glucoside, and 0.2 mg/100 g rutin.

Analysing the microbiological results (Table 4), the EP extracts had a strong inhibitory effect on the growth of microorganisms. EPA samples had lower total viable and yeast/mould counts than the control juice without EP ($P < 0.05$).

Based on the results of the total viable count measurement, at the end of storage, 38% lower value was found in EPA-1 sample and 95% less microbes were detected in EPA-2 juice than in the control juice.

EP extract influenced the yeast and moulds counts over the storage, as control juice had an average of 6 CFU/100 μL, while no yeasts or moulds could be detected in EPA samples.

4. CONLUSIONS

To develop a natural preservative for apple juice products, this study revealed the antioxidant activity and microbial inhibition of elderberry pomace extract when applied for preservation of apple juice.

The FRAP, TPC, and TAC results during storage confirm that the elderberry pomace has an antioxidant effect. There is close correlation between the three parameters, the positive correlation indicates that a significant part of the antioxidant capacity of elderberry is due to the presence of different polyphenols and anthocyanins. The increase in the amount of catechin, epicatechin, and rutin in the samples correlates with the percentage of pomace extract in the apple juice samples. During storage, slight degradation occurred in FRAP and TAC, but the TPC components remained stable.

According to our results, the microbial count was significantly lower at the end of storage compared to the control sample without EPA.

It can be concluded that effective extract possessing strong antioxidant and antimicrobial activity may be obtained from EP; such extracts can be considered as promising additives in apple juice products for improving their microbial stability and significantly enriching them with phytochemicals beneficial to health.

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