Changes in the chemical composition of extracts of wild berries growing in the Republic of Azerbaijan due to enzimatic pretreatment of their pulp

Sevinj Maharramova

Azerbaijan State University of Economics, Baku, Azerbaijan

Keywords:

Wild berries
Maceration
Liquid chromatography
Ascorbic acid
Polyphenols
Heavy metals
Sugars

Article history:

Received 11.06.2023
Received in revised form 18.12.2023
Accepted 29.12.2023

Corresponding author:

Sevinj Maharramova
E-mail: sevinj.mahar@gmail.com

DOI: 10.24263/2304-974X-2023-12-4-5

Abstract

Introduction. The objective of this research was to study the influence of the enzymatic processing of the pulp of dogwood, elderberry, hawthorn, barberry, and raspberry berries on the content of ascorbic acid, sugars, polyphenols and heavy metals in their extracts.

Materials and methods. The berries were processed using enzyme preparations with pectolytic and glycolytic action. The identification of biologically active components in the extracts was carried out using high-performance liquid chromatography with ultraviolet and mass spectrometric detection.

Results and discussion. The use of enzyme preparations increased the yield of juices from berry pulp. The optimal juice extraction was attained after a 120-minute fermentation period for barberry and dogwood berries. For elderberry and raspberry, the optimal periods were 75 and 90 min, respectively. A processing temperature of 45 °C turned out to be the most suitable for most berries, except for barberry, which showed better results at a temperature of 60 °C. The use of both individual enzyme preparations and their complexes had a positive effect on the juice yield for all tested berries. For dogwood and hawthorn, the most effective was the combination of “Pectinex BE XXL” and “Fructosym P”, and for elderberry, barberry and raspberry – “Amylase AG 300L” and “Sellolyuks-A”.

An increase in the concentration of ascorbic acid and sugars in the juices obtained from berries after enzymatic treatment was observed. Enzymatic treatment led to a significant increase of the total concentration of polyphenols, as well as the content of some individual components. Thus, a significant increase in gallic acid content after enzymatic treatment was observed in all samples where it was detected. The exception was coumarins, the concentration of which decreased after enzymatic treatment. It was found that enzyme preparations generally have a positive effect on the content of biologically active substances in the juices of wild berries. However, no significant increase in the concentration of heavy metals was observed. In most cases, the use of multienzyme complexes demonstrated the greatest positive effect.

Conclusions. The results obtained are of high importance for the processing technology of the studied berries showing the promise of the enzymatic maceration for increasing the content of biologically active substances in their juices.

Introduction

Wild berries are actively used in the food industry, including for the production of soft drinks and energy drinks. These raw materials have a high content of biologically active substances, such as polyphenols, vitamins and sugars, which are responsible for the organoleptic and nutritional characteristics of berries (Dhalaria et al., 2020; Nile and Park, 2014; Stabnikova et al., 2024). Several wild berries have a high content of pectin substances, which are used as thickeners and stabilizers to impart the desired consistency and texture to products (Surolia and Singh, 2022). In addition, the use of natural berries has a positive effect on the taste of the product, making it more attractive to consumers. Wild berries are also actively used in the production of natural fruit juices and soft drinks such as lemonades, and other fruit beverages. Due to their high content of natural sugars, vitamins, minerals and antioxidants, wild berries have become popular ingredients in the energy drink industry in recent years.

Wild berries such as dogwood, elderberry, hawthorn, barberry and raspberry are abundant in Azerbaijan and are of interest for commercial juice, as well as puree production due to their high content of beneficial compounds like polyphenols, vitamins and sugars. However, issues like low juice yields and variability in phytochemical content have limited their utilization. Enzymatic pretreatments may help overcome these barriers, but research is lacking on their effects on the composition and properties of juices from these underutilized berries native to Azerbaijan.

To enhance juice yield using enzymatic methods, it is crucial to select enzymes that effectively break down cell walls, facilitating the release of juice. Common choices include pectinases, cellulases, and hemicellulases. These enzymes can be used individually or in combinations, and their selection often depends on the type of fruit and desired characteristics of the juice. Factors like enzyme concentration, temperature, and duration of treatment also play a vital role in maximizing juice yield. Optimizing these parameters through experimental trials and error analysis is essential for achieving the highest possible yield.

In recent years, increasing attention has been paid to the use of enzyme preparations to improve different processes in food technologies. The use of enzymes in the pretreatment of berry pulp can contribute to better extraction of biologically active substances from berry raw materials. Enzymes are capable of destroying cell walls, breaking down complex compounds and increasing the yield of valuable components. M. Kaveh et al. (2020) showed that enzymatic treatment of berries can increase the yield of several biologically active substances by breaking down cell walls. Polyphenols, vitamins, and sugars are of significant value to the food industry, which means increasing the availability of these compounds, which can lead to their increased concentration in the pulp. Polyphenols are biologically active phenolic compounds, widely present in wild berries. According to A.M. Zeynalova et al. (2019) polyphenols isolated from berries growing in Azerbaijan demonstrate antioxidant properties. Meanwhile, it was found that polyphenols exhibit a protective effect on body cells, reducing the risk of developing cardiovascular, oncological and neurodegenerative diseases (Rudrapal et al., 2022). In addition, it was shown that polyphenols have anti-inflammatory and antiviral properties (El-Missiry et al., 2021) and food rich in polyphenols demonstrated anti-obesity effects (Stabnikova and Paredes-Lopez, 2023). The sugar content affects the balance between the sweetness and acidity of the berries, which is important for ensuring a harmonious taste of the drinks, and sugar, found in raw berries, can influence fermentation during the beverage production process (Peighambardoust et al., 2021). Besides that, berries contain a significant amount of vitamins, including ascorbic acid, which exhibit significant antioxidant activity (Davronovich et al., 2022). It is worth noting that wild berries can be contaminated with various environmental pollutants including heavy metals. Such pollution can occur due to industrial emissions, the use of pesticides, soil, and water pollution and several other anthropogenic factors. However, heavy metals such as lead, cadmium, nickel and arsenic are highly toxic to humans, even in trace amounts (Zaynab et al., 2022). Chronic consumption of foods containinghigh amounts of heavy metals can lead to serious problems such as organ damage and nervous system dysfunction. Enzymatic processing of berries can have an impact on the level of heavy metal contamination in theor extracts, so studies such as these will help determine optimal processing conditions that do not increase the content of heavy metals while increasing the concentration of beneficial biologically active substances in the product.

Studying the effect of enzymatic treatment on the content of ascorbic acid, polyphenols, sugars, and heavy metals in berry extracts allows gaining a deeper understanding of the mechanisms and influence of fermentation on the quality, as well as the properties of biologically active components of berries. This may have practical applications for the development of optimal technologies and processes for the production of berry drinks with improved properties, as well as high nutritional value and safety for the consumer.

Thus, the aim of this work was to obtain extracts of wild berries of dogwood, elderberry, hawthorn, barberry and raspberry, to process them with enzymatic preparations with pectolytic and glycolytic effects, as well as to analyse them to study the effect of the enzymatic treatment on the content of ascorbic acid, polyphenols, and sugars in them, as well as heavy metals in berries extracts.

Materials and methods

Samples of wild berries of dogwood (*Cornus mas* L.), elderberry (*Sambucus nigra* L.), hawthorn (*Crataegus monogyna subsp.*), barberry (*Berberis L.*) and raspberries (*Rubus L.*) were collected in various regions of the Republic of Azerbaijan during the period October 2021 to October 2022. Only fully ripe, undamaged berries were selected. Frozen berries were thawed and homogenized. Samples of the resulting pulp weighing 1 kg were heated to a temperature of 50 °C for 8 minutes. After this, enzyme preparations “Pectinex BE XXL”, “Pectinex Yieldash Extra”, “Amylase AG 300 L”, “Fructosym P”, “Rapidaza CR”, “Bryuzaym BGX”, “Laminex super”, “Selloviridin Q20X” were added to the samples. “Sellokyuks-A” and “Enzerzim XT” in amounts of 50, 75, 100 and 150 mg per 1000 g of pulp, as well as their compositions in the following combinations: “Pectinex BE XX” – “Fructosym P” (Multienzyme complex-I), “Amylase AG 300 L” – “Sellokyuks-A” (Multienzyme complex-II) and “Pectinex Yieldash Extra” – “Bryuzaym BGX” (Multienzyme complex-II) in amounts of 50, 75 and 100 mg of each drug per 1000 g of pulp. The maceration process in different experiments lasted 60, 75, 90, 105 and 120 minutes, at temperatures of 30, 45, 60 and 75 °C. Then juices were made from the samples using a Para-press laboratory press (Arauner Kitzingen, Germany) at a pressure of 0.28 MPa for 10 minutes. The results were compared with control samples that were subjected to similar processing but without the step of enzymatic treatment. The most effective enzyme preparation for each type of berry was determined.

To determine the ascorbic acid content, 10 g of raw material was weighed and homogenized using 25 ml of metaphosphoric acid solution with a concentration of 10 g/l. The samples were actively mixed for 15 minutes; after which they were centrifuged at 3800 g for 15 min at 4 °C. The extraction was carried out in duplicate. The resulting supernatants were combined, after which 10 g/l metaphosphoric acid was added, resulting in a volume of 50 ml. A mixture of 1 ml of dithiothreitol with a concentration of 50 g/l and 10 ml of sample was prepared. Metaphosphoric acid was used to bring the volume of solutions to 25 ml. During chromatographic separation, the mobile phases consisted of methanol (phase A) and 0.005 mol/l of KH_2PO_4 solution (pH 2.6, phase B). The gradient method was used as follows: the percentage of phase A was increased from 5% to 22% over 6 minutes, followed by a return to baseline conditions over 9 minutes. Detection was performed at 245 nm and the flow rate 0.7 ml/min. The results were expressed in mg of ascorbic acid per 100 g fresh berry's weight.

To analyse polyphenol content, 10 g of samples were mixed with 50 ml of 70% methanol. The resulting mixture was processed for 15 minutes on a heated shaker and then centrifuged at 3000 g for 20 min. The extraction was repeated twice to ensure full extraction. The collected phenolic extracts were pooled and vacuum evaporated at 40 °C to concentrate them. Distilled water was used to bring the concentrated extracts to a final volume of 25 ml. The mobile phase used for analysis consisted of two components: solvent A, which was prepared by dissolving 60 g of acetic acid in 0.002 mol/l sodium acetate, and solvent B, which was acetonitrile. The entire analysis was carried out for 35 min at a constant flow rate of 1 ml/min. Compound separation was achieved using a gradient program starting with 0-15% B over 15 min, then 15-30% B over 25 min, then 30-50% B over 5 min and finally 50-100% B for 5 min. To quantify phenolic compounds, measurements were carried out at three different wavelengths: 280 nm, 320 nm and 360 nm. An external standard method was used utilizing gallic acid, chlorogenic acid and quercetin as reference standards. Mass spectrometric detection was used to identify polyphenols. Detection was carried out in positive ion mode (Mildner-Szkudlarz et al., 2015).

For sugar analysis, 20 ml of samples were hydrolysed with 1 ml of 1 M sulphuric acid by heating the mixture at 100 °C for 3 hours. After hydrolysis, the resulting hydrolyses were diluted 20-fold with distilled water and then treated with 1-phenyl-3-methyl-5-pyrazolone (PMP) for derivatization. As an internal standard, 10 µl of 0.5 mM 2-deoxyglucose was added to each sample. Sugar separation was achieved at a flow rate of 0.6 ml/min and was carried out at 30 °C. The mobile phases used for separation consisted of two components: (A) a mixture of 10% acetonitrile and 40 mM ammonium acetate at pH approximately 6.8 and (B) 70% acetonitrile. The separation process followed a gradient elution starting with 8% phase B, which increased to 16% phase over 12 min. Detection was performed at 250 nm. To determine the sugar content, the areas under the peaks observed in the chromatograms were compared with standard samples of glucose, fructose, galactose, and sucrose. All chromatographic studies were carried out using an Agilent Technologies 1200 LC system (Agilent Technologies, USA) equipped with a UV detector and a mass spectrometer. Separation was carried out on a Poroshell 120, SBC18 column (4.6 x150 mm, 2.7 µm). The concentration of a number of heavy metals was also measured in the resulting juices. Ni, Cu, Co, Pb, Cd and As were determined by atomic absorption spectrometry on a Perkin-Elmer 3030B flame spectrometer. Cr and Sr were analysed using an ISP AtomScan 25 emission spectrometer.

Results and discussion

When conducting preliminary experiments to measure the yield of juice from dogwood, elderberry, hawthorn, barberry and raspberry berries, the most effective enzyme preparations and optimal processing modes were established. The highest juice yield for barberry and dogwood berries was observed during fermentation for 120 minutes. For elderberry and raspberry, the optimal period was 75 and 90 minutes, respectively. With longer processing for these berries, no significant increase in juice yield was observed. For most of the berries tested, the optimal processing temperature was 45 °C. The exception was barberry berries, for which this parameter was 60 °C.

The results obtained indicate that the most effective of the studied preparations for dogwood berries was the enzyme “Fructosym P” when treated, with which the juice yield increased from 50.2±1.34% to 58.5±0.78%. For elderberry and barberry, the enzyme preparation “Pectinex BE XXL” turned out to be optimal; when used, the juice yield increased from 54.5±0.87% and 52.6±2.24% to 61.3±1.74% and 60.4±3.08%, respectively. When processing hawthorn, the most effective enzyme was “Amylase AG300L”; compared to control samples, the juice yield increased from 48.4±1.06% to 57.8±1.56%. For raspberries, the enzyme preparation “Pectinex Yieldash Extra” was the most effective; after treatment, the juice yield increased compared to control samples from 58.4±1.46% to 67.2±3.23%. When processing dogwood and hawthorn, the most effective was the multienzyme complex consisting of the preparations “Pectinex BE XXL” and “Fructosym P”, the juice yield increased from 50.2±1.34% and 48.4±1.06% to 64.6±3.04% and 60.5±2.54% respectively. For elderberry, barberry and raspberry, the combination of drugs “Amylase AG 300L” and “Sellolyuks-A” demonstrated the greatest efficiency, while the juice yield increased from 54.5±0.87%, 52.6±2.24% and 58.4±1.46% to 66.3±1.73%, 65.8 ±3.18% and 69.4±1.35%, respectively.

It was found that preliminary enzymatic treatment of crushed dogwood, elderberry, hawthorn, barberry, and raspberry berries with various enzyme preparations helps to increase the yield of unclarified juices by an average of 6.8-9.4% compared to control samples. The most effective drugs are “Fructosym P”, “Pectinex BE XXL” and “Amylase AG 300L”. When using the most effective multienzyme complexes, the juice yield increased by an average of 11-14.4% compared to control samples and by 2.2-6.1% compared to the use of effective enzymes. It was shown that the use of a complex of preparations “Pectinex BE XXL” and “Fructosym P” was more effective when processing dogwood, with an increase in juice yield from which was 6.1%. When processing elderberry and barberry, the best result was demonstrated by the mixture of Amylase AG 300L and Sellolyuks-A: the increase in juice yield was 5 and 5.4%, respectively. The use of multienzyme complexes turned out to be relatively less effective for hawthorn and raspberry, as juice yield increased by only 2.7% and 2.2%, respectively.

The concentration of ascorbic acid was measured in dogwood, elderberry, hawthorn, barberry, and raspberry berries subjected to preliminary enzymatic treatment, as well as without such treatment. The obtained data are presented in Table 1.

The yield of vitamin C in the juice fraction after treating the berries with the most effective enzyme preparations increased for dogwood berries by 1.18 times, elderberries by 1.23 times, hawthorn by 1.22 times, barberries by 1.38 times, and raspberries by 1.2 times. An increase in the content of ascorbic acid in the juices of these berries using multienzyme complexes was observed on average by 1.35-1.52 times. The most likely reason for the increase in the yield of ascorbic acid is the hydrolytic breakdown of the structural components of the cell wall, and above all, cellulose and hemicellulose, as a result of which bound forms of vitamin C are released into juices. In the analysed juices, the presence of glucose, fructose, galactose, and sucrose were identified and the concentrations were quantified. The results are shown in Table 2.

Table 1

Content of ascorbic acid in berry juices subjected to various types of preliminary enzymatic maceration

Berry	**Ascorbic acid concentration (mg/100 g)**				
	No treatment	**Best drug**	**Multienzyme complex**	**“No treatment” vs. “Best drug” (paired t-test) (Not statistically significant at α = 0.05)**	**“No treatment” vs. “Multienzyme complex” (paired t-test) (Not statistically significant at α = 0.05)**
Dogwood	75	88.7	101.2	p-value = 0.057	p-value = 0.004
Elderberry	38.2	47.2	52.4	p-value = 0.086	p-value = 0.001
Hawthorn	39.8	48.6	57	p-value = 0.095	p-value = 0.0002
Barberry	84.5	116.8	128.3	p-value = 0.001	p-value = 0.00005
Raspberry	57.6	69.1	81.3	p-value = 0.002	p-value = 0.00002

Source: compiled by the author

Table 2

Sugar concentration in berry juices subjected to various types of preliminary enzymatic maceration

Berry	**Processing method**	**Content (g/100g)**			
		glucose	**fructose**	**galactose**	**sucrose**
Dogwood	No treatment	4.2	3.8	0.7	0.5
	Best drug	5.2	4.1	0.8	0.6
	Multienzyme complex	6.5	5.7	1.3	0.8
Elderberry	No treatment	5.7	4.6	0.5	0.3
	Best drug	6.7	4.5	0.6	0.4
	Multienzyme complex	6.9	7.1	0.6	0.7
Hawthorn	No treatment	8.5	3.4	0.2	1.5
	Best drug	8.7	4.8	0.3	1.4
	Multienzyme complex	9.4	7.8	0.3	1.7
Barberry	No treatment	7.3	6.5	0.1	0.1
	Best drug	8.1	7.8	0.1	0.2
	Multienzyme complex	9.7	8.9	0.2	0.4
Raspberry	No treatment	7.6	6.8	0.2	0.4
	Best drug	8.2	6.9	0.5	0.5
	Multienzyme complex	8.3	7.4	0.5	0.6

Source: compiled by the author

Because of treating the pulp with enzyme preparations, a significant increase in glucose concentration was observed in all studied samples, which was directly related to the breakdown of polysaccharides. In addition, increases of the amounts of other sugars were also observed. This effect was most significant when using multienzyme complexes. When using the enzyme preparation “Fructosym P”, the increase in sucrose content in the samples was on average no more than 12%, and when using the complex of preparations “Pectinex

BE XXL” and “Fructosym P”, an increase in the concentration of this component was observed by 1.58 times. In general, the increase in the concentration of sugars in the samples is associated with the destruction of the cell wall, and s a result of what their extractability increases. The total concentration of polyphenols in berry juices is given in Table 3.

Table 3

Total content of phenolic compounds in berry juices subjected to various types of preliminary enzymatic maceration

Berry	**Ascorbic acid concentration (mg/100 g)**		
	No treatment	**Best drug**	**Multienzyme complex**
Dogwood	645.1	657.5	705.3
Elderberry	575.8	596.8	613.6
Hawthorn	458.9	499.3	511.1
Barberry	589.2	610.1	610.9
Raspberry	35.8	46.2	56.5

Source: compiled by the author

The total content of phenolic compounds was highest in the juices of dogwood berries. A significant increase in the total concentration of polyphenols in pulp samples was observed after their treatment with enzymatic preparations. The data obtained indicate that the destruction of polysaccharides during enzymatic maceration leads to the release of some polyphenols. During the study, 10 phenolic compounds were identified in the analysed pulp extracts. However, only coumarins, quercetin, gallic and chlorogenic acids were measured quantitatively.

The largest amount of phenolic compounds was found in dogwood berries. Its juices, pre-treated with enzyme preparations, contained chlorogenic acid, 257 mg/100 g, and quercetin, 65 mg/100 g. It was found that the content of the latter in dogwood and elderberry juices prepared from pulp subjected to pectinolysis was higher than in untreated samples by 15% and 17%, respectively. In all samples where gallic acid was detected, a significant increase in its content was observed after enzymatic treatment. Compared to untreated samples, hawthorn juices prepared from pulp subjected to pectinolysis showed the most significant increase by 16%. Several samples showed the appearance of peaks of certain compounds after enzymatic hydrolysis. The only exception was hawthorn juice, the peak of quercetin in which disappeared after treating the samples with enzymatic preparations. Among the hydroxybenzoic acids, two compounds have been identified: 4-hydroxybenzoate and formyl salicylic acid. It is worth noting that the content of 4-hydroxybenzoate increased significantly in samples subjected to enzymatic maceration. 4-Hydroxybenzoate is a phenolic compound associated with plant cell walls, which may explain its higher content in pre-treated extracts. The data obtained indicate that the use of enzymatic treatment of pulp may increase the extractability of 4-hydroxybenzoate. However, the biosynthetic pathway and degradation of 4-hydroxybenzoate in plants are not yet fully understood.

The evaluation of juices' organoleptic properties entails the assessment of sensory attributes such as flavour, fragrance, visual appeal, and consistency. To assess the sensory properties of dogwood, elderberry, hawthorn, barberry, and raspberry juices produced through enzymatic treatment, a descriptive approach was used for organoleptic evaluation (Table 4).

Table 4

Organoleptic evaluation of juices

Berry	Appearance	Aroma	Taste
Dogwood	The juice has a vibrant reddish hue with excellent clarity, making it visually appealing.	The juice offers a delicate and fruity aroma with hints of berry notes and a subtle floral undertone.	The taste profile is pleasantly sweet, mildly tart, and features distinct berry-like flavors.
Elderberry	The juice exhibits a deep purple color with good clarity, which is visually striking.	The juice releases a strong and distinctive berry fragrance, displaying the characteristic aroma of elderberries.	The juice offers a well-balanced sweetness with a noticeable tartness and a rich, bold berry flavor.
Hawthorn	The juice is slightly cloudy with a pleasant, pale red color.	The juice has a mild, fruity aroma with faint floral hints that contribute to its overall appeal.	The juice has a mildly sweet and slightly tart taste with subtle fruity notes.
Barberry	The juice is clear and possesses an inviting golden color.	The juice has a fresh and slightly tangy aroma with subtle citrus notes, reflecting its unique character.	The juice is characterized by its refreshing tartness, mild sweetness, and a unique citrus-like tang.
Raspberry	The juice is notably vivid, displaying a rich red hue that is visually enticing.	The juice presents a bold and fruity aroma dominated by the sweet and tangy scent of ripe raspberries.	The juice is notably sweet, tangy, and bursting with the authentic flavor of ripe raspberries.

Note: This organoleptic evaluation is subjective and reflects one individual's assessment.
Source: compiled by the author.

Furthermore, an examination of the overall characteristics of these wild berries is included: (a) the consistency of all the juices is velvety and devoid of any discernible pulp, ensuring a delightful drinking sensation; (b) elderberry and raspberry juices stand out for their rich and distinctive flavors, with elderberry offering a berry profile, and raspberry providing an authentic raspberry experience. Dogwood juice also deserves recognition for its balanced sweetness and fruity appeal.

Among the derivatives of hydroxycinnamic acid, two compounds have been identified: chlorogenic acid and coumarin. Chlorogenic acid content showed no significant differences between fermented samples and extracts obtained from untreated pulp. Elderberry juices subjected to enzymatic treatment had the highest content of chlorogenic acid. The content of coumarins was significantly lower in juices prepared from unprocessed pulp. However, overall, the results of the study indicate that enzymatic treatment of berry pulp led to better retention of phenolic compounds in juices compared to untreated samples.

Data regarding the content of heavy metals in the analysed juices are presented in Table 5.

Table 5

Concentration of heavy metals in juices of berries subjected to various types of preliminary enzymatic maceration

Berry	Processing method	Content, mg/100 g							
		Ni	Cu	As	Cd	Co	Cr	Pb	Sr
Dogwood	No treatment	1.71	1.33	0.04	-	0.01	0.05	0.24	0.2
	Best drug	1.74	1.34	0.04	-	0.01	0.05	0.24	0.2
	Multienzyme complex	1.73	1.4	0.04	-	0.01	0.05	0.25	0.21
Elderberry	No treatment	0.62	0.81	0.03	-	-	0.04	0.15	0.09
	Best drug	0.64	0.82	0.03	-	-	0.04	0.15	0.09
	Multienzyme complex	0.66	0.85	0.03	-	-	0.04	0.16	0.09
Hawthorn	No treatment	1.23	1.2	-	-	0.01	0.05	0.16	0.1
	Best drug	1.24	1.21	-	-	0.01	0.05	0.16	0.1
	Multienzyme complex	1.29	1.26	-	-	0.01	0.05	0.17	0.11
Barberry	No treatment	1.47	1.13	0.10	-	0.07	0.03	0.05	0.07
	Best drug	1.48	1.14	0.10	-	0.07	0.03	0.05	0.07
	Multienzyme complex	1.54	1.19	0.11	0.01	0.07	0.03	0.05	0.07
Raspberry	No treatment	0.35	0.54	0.01	-	0.02	0.03	0.1	0.03
	Best drug	0.35	0.55	0.01	-	0.02	0.03	0.1	0.03
	Multienzyme complex	0.37	0.57	0.01	-	0.02	0.03	0.11	0.03

Source: compiled by the author

The results demonstrate that the methods of preliminary enzymatic maceration used do not lead to a significant (more than 5%) increase in the concentration of heavy metals in the juices of the tested berries. It is worth noting that cadmium, Cd, was not found in any of the unprocessed juices. A low concentration of this metal (0.01 mg/100 g) was recorded only in barberry juices treated with a multienzyme complex. Cobalt, Co, was not found in elderberry juices, and arsenic, As, was not found in hawthorn juices.

Discussion

The obtained results showed a notable enhancement in the juice yield from berries following their pre-treatment with enzyme preparations. The used pectolytic preparations are capable of destroying pectins, which are the main component of cell walls, which makes them more susceptible to mechanical destruction. L.R. Larsen et al. (2021) showed that this process facilitates the release of juice during further processing of berries. Also, it was demonstrated that preliminary enzymatic maceration promotes better drainage of juice from berries. This is due to their ability to break down cellular structures and facilitate fluid flow. Thanks to this, the process of juice extraction becomes more complete and efficient. Enzyme preparations with glycolytic action can soften the cells and tissues of berries (Streimikyte et al., 2022). They affect glycosidic bonds in carbohydrate molecules, which leads to the destruction of cell structure. Softening the tissue makes it easier to release the juice by pressure or centrifugation.

Enzyme preparations may affect oxidases that are present in the samples, which may promote the oxidation of ascorbic acid. C. Bender et al. (2017) reported a similar effect in their study, where they found that heating blackcurrant pulp before pectinolysis resulted in higher ascorbic acid content in juices compared to pectinolysis without heating. However, this effect was species-dependent, since, for example, it was not observed in raspberries. Similarly, a decrease in ascorbic acid content in goldenrod berry extract prepared from enzymatically treated pulp was not observed in the study of M.F. Ramadan and H.T. Moersel (2006). Long-term ageing of barberry pulp with preliminary enzymatic and thermal treatment led to a decrease in the content of ascorbic acid in the extracts and juices prepared from enzymatically treated raw materials had significantly lower ascorbic acid content compared to untreated samples (Radziejewska-Kubzdela et al., 2020). It was suggested that the retention of ascorbic acid during pretreatment without prior inactivation of ascorbic acid oxidase might depend on the specific profile of phenolic compounds present in the raw material (Clegg and Morton, 1968). In this study, no reduction in vitamin C content was observed in the samples due to enzymatic treatment. On the contrary, there was a noticeable increase in the concentration of ascorbic acid in juices treated with multienzyme complexes.

As with other biologically active substances, one of the main mechanisms for increasing polyphenol concentrations is likely due to breakdown by cell wall enzymes. Enzymatic pre-treatment helps release polyphenols, making them easier to extract and increasing their concentration in the extract. In addition, introduced enzymes that can activate internal enzymatic systems such as polyphenoloxidases present in berries (Nicolescu et al., 2022). These enzymes catalyse the oxidative reactions of polyphenols, which can lead to the formation of polymeric and oxidized forms. In addition, enzymes can promote the hydrolysis of glycosidic bonds that link polyphenols to other molecules such as sugars or organic acids. This may result in the release of polyphenols from complexes and increase their availability for extraction. The use of various enzymes (Cellubrix, Neutrase and Viscozyme) on grape pomace from Cabernet (red) and Garnatxa (white) varieties increased the yield of antioxidant compounds during extraction (Costoya et al., 2010). Enzymatic treatment, especially when a mixture of three enzymes was used, significantly increased extractable phenolics by 21% for Garnatxa and 46% for Cabernet compared to untreated samples. At the same time, enzymatic treatment also increased the solubility of several compounds in water. This suggests that administering enzymatic drugs prior to treatment may enhance the ability of compounds to dissolve, thereby potentially improving their availability for biological processes and the efficiency of their extraction.

It was found that because of the pre-treatment of berries with enzyme preparations, a more effective extraction of polyphenols and an increase in their concentration in the extract is achieved. The findings are consistent with previous studies that also reported similar effects. The study conducted by L.N. Lieu and B.B. Le (2010) compared the effects of enzymatic treatment and sonication on grape must. An increase in the overall concentration of phenolic compounds in the berry extracts, even after enzymatic treatment was observed. However, in a study of E. Radziejewska-Kubzdela et al. (2020), no such relationship was found. On the contrary, they observed a decrease in the total amount of polyphenols in berry extracts because of pre-enzymatic treatment. They suggested that the effect might be due to the activity of a by-product esterase present in the enzyme used, or to partial degradation of phenolic compounds during processing. In addition, residual polyphenol oxidases present in grape pomace may also contribute to the reduction of phenolic and antioxidant content. Researchers demonstrated that the use of enzymatic or thermal pretreatment improved the extractability of 4-hydroxybenzoate compared to sonication or untreated samples. The specific mechanisms underlying this improvement may vary and include factors such as increased enzymatic activity, cell wall disruption, or increased solubility of 4-hydroxybenzoate during enzymatic pretreatment processes. A. Chaovanalikit and R.E. Wrolstad (2008) suggest that enzyme preparations may promote the activation of endoglycosidases, which hydrolyse glycosidic bonds connecting phenolic compounds to other molecules, in particular sugars. As a result, this causes the anthocyanins to be released more and become more extractable. In addition, according to E. Radziejewska-Kubzdela et al. (2020), the activity of pectolytic enzymes can affect the activity of polyphenol oxidase, which oxidizes several polyphenols.

During the study, it was shown that pre-treatment of berries with enzyme preparations of pectinase and cellulose leads to an increase in the concentration of sugars in their extracts. One of the main mechanisms for increasing the concentration of sugars is associated with the destruction of cell wall polysaccharides. Pre-treatment with pectolytic enzymes promotes the breakdown of these polysaccharides into mono and disaccharides, mainly glucose and fructose. According to K.R. Corbin et al. (2015), this process also increases the availability of sugars for extraction and leads to their increased concentration in the extract. Researchers claim that pectolytic enzymes can activate glycosidases, which hydrolyse the glycosidic bonds connecting carbohydrates to glycoproteins, which also contributes to the release of monosaccharides and their increased concentration in the extract. In addition, they suggested that pretreatment with enzyme preparations may also promote the activation of endogenous amylases, which break down oligosaccharides into mono and disaccharides. In a study by the researchers, the decrease in soluble and insoluble dietary fibre and the increase in free monosaccharides following enzymatic hydrolysis of fruit peels is consistent with the breakdown of complex polysaccharides into simpler sugars. This study demonstrated that after pre-treatment of berries with enzyme preparations, the concentration of sugars in the extracts increases. Thus, the results obtained are mainly consistent with those obtained from the literature.

The obtained values of concentrations of heavy metals in the juices of wild berries correlate with the results obtained by L.F. Steingräber et al. (2021). The researchers' data indicate that drugs used in the enzymatic processing process can potentially activate the processes of mobilization of heavy metals from berry tissues. If the berries have been contaminated with heavy metals from the environment, enzymes can help release those metals from the cells, making them more available for extraction in the juice. However, in this study, no significant increase in the content of heavy metals was observed after enzymatic treatment, which may be due to the initially low content of these components in the analysed berries.

Conclusions

1. This study examined the impact of treating wild berry pulp with enzyme preparations on the concentration of biologically active compounds in their juices. The optimal juice extraction was attained after a 120-minute fermentation period for barberry and dogwood berries. For elderberry and raspberry, the optimal periods were 75 and 90 min, respectively. A processing temperature of 45 °C turned out to be the most suitable for most berries, except for barberry, which showed better results at a temperature of 60 °C.

2. The use of both individual enzyme preparations and their complexes had a positive effect on the juice yield for all tested berries. For dogwood and hawthorn, the most effective was the combination of "Pectinex BE XXL" and "Fructosym P", and for elderberry, barberry and raspberry – "Amylase AG 300L" and "Sellolyuks-A". The yield of vitamin C increased for dogwood berries – 1.18 times, for elderberries – 1.23 times, for hawthorn – 1.22 times, for barberry – 1.38 times, and raspberries – 1.2 times. In most cases, the use of multienzyme complexes demonstrated the greatest positive effect.
3. The use of multienzyme complexes additionally increased the vitamin C content in the juices of these berries by an average of 1.35-1.52 times.
4. Juices after treatment with enzymes showed a significant increase in sugar content; the greatest effect was achieved when using multienzyme complexes. The complex "Pectinex BE XXL" and "Fructosym P" increased the concentration of sucrose in the samples by an average of 1.58 times.
5. It was found that enzyme preparations generally have a positive effect on the content of biologically active substances in the juices of wild berries. Dogwood berry juices showed the highest amount of phenolic compounds among the tested samples. After treatment with enzyme preparations, they contained a predominant amount of chlorogenic acid (257 mg/100 g) and quercetin (65 mg/100 g). The quercetin content in pre-treated dogwood and elderberry juices was higher by 15% and 17%, respectively, compared to untreated samples. A significant increase in gallic acid content after enzymatic treatment was observed in all samples where it was detected. Hawthorn juices showed the most significant increase in the content of this component (16%).
6. No significant influence of enzymatic pretreatment on the concentration of heavy metals in berries extracts was found.
7. The results of the study indicate the promise of enzymatic maceration of wild berries for their further use in the food industry. Further research is needed to establish in more detail the mechanisms of such effects for different classes of compounds.

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