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The Influence of Pre-Treatment of Grape, Cherry, and Strawberry Pulp with Enzyme Preparations of Pectinase and Cellulase on some Organic Compounds Amount in their Extracts

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Berry crops play a significant role in Azerbaijan's agricultural sector. Pre-processing berry pulp with enzyme preparations can enlarge the quantity of some useful organic molecules in their extracts, such as vitamins, anthocyanins, polyphenols, and monosaccharides. These compounds are highly valuable for the food industry, and enhancing their concentration is a relevant issue. The current investigation aimed to study the effect of the enzymatic maceration process on the pulp of grapes, cherries, and strawberries on the content of the aforementioned substances in their extracts. The berries were treated with pectinase and cellulase preparations. The bioactive components in homogenate were determined and separated using high-performance liquid chromatography, with ultraviolet and mass spectrometric detection. A slight increase in the concentration of ascorbic acid was observed in the obtained extracts. Several anthocyanins and polyphenols were successfully identified in the investigated berries. Enzymatic treatment caused a sufficient increase in their overall concentration, as well as the content of some individual components. Coumarins were an exception, as their concentration decreased due to enzymatic treatment. It was demonstrated that the use of pectinase and cellulase preparations increased not only the glucose but also other monosaccharides' content. The combination of two enzyme preparations had the greatest positive effect on the monosaccharide content in the investigated extracts. The obtained data are of great importance for the food industry regarding the studied berries and indicate the potential of enzymatic maceration as a method for increasing the content of bioactive components in grape, cherry, and strawberry products.

Keywords: anthocyanins, pectinolysis, cellulases, monosaccharides, polyphenols

Introduction

Berry crops, such as cherries, grapes, and strawberries, are essential for the agricultural sector of the Republic of Azerbaijan. Grapes are one of the most important berry crops in Azerbaijan, cultivated for fresh berries, raisins, winemaking, and the production of canned products. Strawberries and cherries are also popular berry crops for both domestic consumption and export. These crops play an important part in the agricultural sector of the Republic of Azerbaijan, providing income to farmers, creating job opportunities, diversifying food resources, and offering additional export possibilities. Therefore, cherries, grapes, and strawberries are of substantial importance for the economic and social development of the country.

E. Radziejewska-Kubzdela et al. [1] demonstrated that the enzymatic processing of berries could contribute to the breakdown of cell wall polysaccharides, releasing vitamins, polyphenols, and anthocyanins. According to the results obtained by S. Aliyev et al. [2], fruit pectinolysis contributes to the increased content of bioactive substances in their products. These bioactive components hold high value for the food industry, making the task of increasing their availability and, consequently, possible concentration in pulp relevant. Polyphenols are biologically active phenolic compounds widely present in grapes, cherries, and strawberries, including in grapefruits. According to the findings of E. Radziejewska-Kubzdela et al. [1], polyphenols exhibit significant antioxidant properties, neutralizing free radicals and reducing oxidative stress levels in the body.

R. Khoshamad et al. [3], M. Gavahian and C. Hsieh [4] noted a sufficient concentration of phenol derivatives in grape and strawberry berries, which were known as antioxidants. A. Chaovanalikit and R.E. Wrolstad [5] showed that flavonoids are the main class of polyphenols found in cherries. Research by M. Rudrapal et al. [6] demonstrated that polyphenols have a protective effect on the body, reducing the likelihood of cardiovascular, oncological, and

neurodegenerative diseases. Additionally, scientists, including M.A. El-Missiry et al. [7], proved that polyphenols have anti-inflammatory and antiviral properties, contributing to maintaining optimal health. Anthocyanins are another class of water-soluble flavonoid molecules that determine the dye of fruits. According to M. Babashpour-Asl and M. Piryaei [8], grape anthocyanins exhibit pronounced antioxidant properties.

J. Chen et al. [9] showed that anthocyanins display anti-inflammatory activity, contribute to strengthening immunity, and decrease the possibility of the development of certain types of tumours. W. Kalt et al. [10] demonstrated that they reduce the likelihood of cardiovascular diseases and contribute to improved cognitive functions. Furthermore, the aforementioned berry crops also contain a significant number of vitamins, including ascorbic acid, which, according to E. Radziejewska-Kubzdela et al. [1], also exhibits substantial antioxidant properties. Following M. Majdan and B. Bobrowska-Korczak [11], grape berries contain a significant number of monosaccharides such as glucose, galactose, xylose, and arabinose, while strawberries and cherries have a high fructose content. Besides their energetic function, the sugar content characterizes the gastronomic and technological value of these crops.

Studying the impact of enzymatic processing on the ascorbic acid, polyphenols, anthocyanins, and monosaccharides concentration in fruit homogenate contributes to a more profound understanding of the fermentation's influence on the quality and properties of bioactive components in berries. This has potential applications for developing optimal technologies and production processes for berry products with enhanced properties and nutritional value. This study aimed to obtain extracts from different grape, cherry, and strawberry varieties, subject them to enzymatic processing using pectinase and cellulase, and conduct chromatographic analysis to study the effect of the treatment on the ascorbic acid, polyphenols, anthocyanins, and monosaccharides quantities in them.

Materials and Methods

Samples of grapes (*Vitis vinifera*) of the "Pinot noir" and "Aligot" varieties, cherries (*Prunus cerasus*) of the "Ramon Oliva" and "Biraggo Grohl" varieties, as well as strawberries (*Fragaria viridis*) of the "Albion" and "Victoria" varieties, were provided from the experimental base of the Scientific Research Institutes of Viticulture and Winemaking and Fruit and Tea Growing of the Ministry of Agriculture of the Republic of Azerbaijan. Only fully ripened and undamaged berries were selected. Strawberry and cherry harvesting took place in June, while grape harvesting occurred in September 2022. To preserve the quality of the berries, they were immediately frozen at -30 °C. At the start of the experiment, the frozen berries were thawed and homogenized. In total, 1 kg of the obtained pulp was taken and treated at 50 °C for 8 minutes. Afterward, pectinase (Rohapect 10L, AB Enzymes, Germany) and cellulase from *Aspergillus niger* (Sigma-Aldrich, USA) were added to the samples at 0.3 g per 1000 g of pulp, as well as a mixture of both enzymes at 0.3 g each per 1000 g of pulp. The maceration process lasted for 60 minutes, adhering to the parameters recommended by the manufacturer. Following the enzymatic pretreatment, the samples were pressed using a laboratory Para-press (Arauner Kitzingen, Germany) at a pressure of 28 bar for 600 seconds. The obtained material has been kept at -30°C. The results were compared with control samples that underwent similar sample preparation but without the addition of pectinase.

To determine the content of ascorbic acid, 10 g of the fruit mass was dissolved in 0.025 L of HPO_3 (1%) during 0.25 h with shaking and precipitated at 4800 rot/min for 0.25 h at 4°C. To get the final sample, a double extraction has been performed. All the solution after sedimentation has been additionally treated with HPO_3 (1%) in a total volume of 0.05 L. A solution of 1 ml of dithiothreitol (5%) and 0.01 L of the sample was prepared. To bring the sample volume to 25 ml, metaphosphoric acid was added. Chromatographic separation used methanol (Phase B) and a solution of KH_2PO_4 with a concentration of 5 mmol per 1 L (pH 2.6, Phase A). A gradient method was employed, gradually increasing the content of Phase B from 5% to 22% over 6 minutes (700 mcl/min), with subsequent column equilibration at 5% Phase B over 9 minutes. The signal of the detector was monitored at 245 nm. The calculation was presented as the amount of ascorbate (mg) in 0.1 kg of raw fruit.

For anthocyanin analysis, 10 g of the sample was homogenized and extracted with a solvent mixture consisting of $CH_3COOH/H_2O/CH_3OH$, in a ratio of 2:48:50 by volume. Obtained eluates were shaken for 0.25 h and precipitated at 3000g for 0.33 h. This extraction process was repeated. The solutions were evaporated at 35°C under a vacuum (Büchi R-205, Flawil, Switzerland). The concentrated anthocyanin extracts were dissolved in water and subjected to chromatographic separation. A solvent A consisting of 10% HCOOH; solvent B included water, HCOOH, and acetonitrile in a ratio of 10:60:30. A method was employed: 0-8 minutes with 80% A, 8-15 minutes with 60% A, 15-16 minutes with 40% A, and 16-20 minutes with 100% B with a velocity of 1000 µl per min and scanning wavelengths from 250 to 600 nm. The total amount of anthocyanin was measured by mass (mg) of cyanidin-glucoside in 0.1 kg of raw fruit (w.m.).

For polyphenol content determination, 0.01 kg of fruit mass was taken and homogenized with 0.05 L of methanol (70%). The methanol extracts were shaken for 0.25 h in a shaker with heating and then centrifuged at 3000g for 20 minutes. The described procedure was doubled. The obtained phenolic extracts were

dried under vacuum at 40°C and condensed to 0.025 L with H_2O. Phase A included 6% CH3COOH and 2 mM NaCl in water. Phase B was absolute acetonitrile. Chromatographic elution performed in conditions: 100-85% A over 0.25 h, 85-70% A over 0.42 h, 70-50% A over 0.08 h, and 50-0% A over 0.08 h with a velocity of 1000 µl per min during 0.58 h. Phenolic compounds were quantitatively determined at 360 nm, 320 nm, and 280 nm by normalization with quercetin, chlorogenic, and gallic acids as standards. Mass spectrometric detection was used for phenol identification, performed in positive ion mode by analogy of methodology by S. Mildner-Szkudlarz et al. [12].

To perform monosaccharide analysis, 20 mg samples were hydrolyzed at 100 °C for 180 min, using 1 ml of 1 M H_2SO_4. The resulting hydrolysates were diluted 20 times with distilled water and derivatized using 1-phenyl-3-methyl-5-pyrazolone (PMP). 10 µl of 500 µM 2-deoxyglucose was added to each sample (standard). Monosaccharides were separated with a velocity of 600 µl per min at 30°C. The mobile phase A included 0.04 M CH_3COONH_4 (pH~6.8) with 10% acetonitrile. The mobile phase B included 70% acetonitrile in the same buffer. Gradient elution started with 8% of phase B and increased to 16% of phase B over 12 minutes. The analytes were screened at 250 nm. To determine the concentration of monosaccharides, the areas under the peaks on chromatograms were compared with standards for glucose, fructose, galactose, xylose, arabinose, and mannose. All chromatographic studies were made with the Agilent Technologies 1200 system, manufactured by Agilent Technologies in the USA. This system included a UV detector and a mass spectrometer. For Separation was performed by Poroshell 120-SBC18 column (4.6×150 mm; 2.7 µm).

Results

As a result of the work, the ascorbate amount was determined in grape, cherry, and strawberry, subjected to pre-treatment with pectinase, as well as without such treatment. The obtained results are demonstrated in Table 1.

According to Table 1, the content of vitamin C has not been critically changed in berry pulp depending on their treatment with the enzyme preparation pectinase. However, the concentration of ascorbate in samples treated with a mixture of enzyme preparations exceeded the corresponding indicator in untreated samples for all the cultures studied. The highest vitamin C content was demonstrated by samples of strawberries of the “Victoria” variety treated with both enzyme preparations. There was also some noticeable enlargement of the amount of ascorbate for strawberries of the “Albion” variety treated with pectinase preparations, as well as a mixture of two enzyme preparations. The total anthocyanins quantity in the samples of the studied crops is presented in Table 2.

Table 1. Content of ascorbic acid in berry extracts subjected to various types of preliminary enzymatic maceration.

Culture	Ascorbic acid concentration (mg/100 g)			
	No processing	Pectinase	Cellulase	Pectinase and cellulase
Grapes (variety “Pinot noir”)	3.6	3.4	3.4	3.7
Grapes (variety “Aligot”)	4.4	4.7	5.1	5
Strawberries (variety “Albion”)	61.7	67.1	63.8	68.3
Strawberries (variety “Victoria”)	74.9	78.2	77.1	78.4
Cherry (variety “Ramon Oliva”)	8.3	9.8	7.4	10.1
Cherry (variety “Biraggo Grohl”)	15.7	16.1	14.9	16.5

Table 2. Total anthocyanin amount in berry extracts subjected to various types of preliminary enzymatic maceration.

Culture	Total anthocyanin content (mg/100g)			
	No processing	Pectinase	Cellulase	Pectinase and cellulase
Grapes (variety “Pinot noir”)	214.3	234.7	267.4	298.9
Grapes (variety “Aligot”)	5.8	7.4	6.1	7.8
Strawberries (variety “Albion”)	74.1	76.2	79.9	85.4
Strawberries (variety “Victoria”)	77.4	88.3	87.2	90.5
Cherry (variety “Ramon Oliva”)	142.7	159.9	167.5	170.2
Cherry (variety “Biraggo Grohl”)	97.8	116.2	114.8	126.6

The highest overall number of anthocyanins was observed in the "Pinot noir" samples. At the same time, the lowest was found in the grapes of the "Aligot" variety. For all analyzed samples, there was a sufficient increment of anthocyanins after treating the pulp with enzymatic preparations. The most substantial increase in anthocyanin content was demonstrated in the grapes of the "Pinot noir" variety. In the case of the combination of pectinase and cellulase enzymatic preparations, it reached almost 140 % of the corresponding pulp extract that did not undergo pretreatment. A less pronounced effect was observed for the "Aligot" grapes and "Albion" strawberries. In the case of grapes, the low overall anthocyanin content might be the cause of this effect. For strawberries, the results could be associated with the initially high availability of these substances, making the breakdown of cell walls almost ineffective in releasing additional anthocyanins. Selective specific identification and quantitative measurement of some detected anthocyanins were performed. Among the anthocyanins in the "Pinot noir" grapes, delphinidin 3-monoglucoside and malvidin 3-monoglucoside predominated. Enzymatic treatment of the grapes' pulp of this variety increased the malvidin 3-monoglucoside content in the pulp extracts by 20 % compared to its content in untreated samples. Pulp extracts subjected to pectinolysis contained delphinidin 3-monoglucoside comparable to that in untreated samples. As for 3-monoglucoside-acetate, only the treatment of the pulp with cellulase and a combination of two enzymatic preparations caused an essential augmentation (2.5 times) in its content in the juice compared to untreated samples. The quantity of other identified anthocyanins in the analyzed extracts did not show significant differences. The observed enlargement of the concentration of some anthocyanins in pulp extracts after prior enzymatic processing can be explained by their specific profile. The higher content of anthocyanins obtained from enzymatically treated pulp indicates an improvement in the extractability of these compounds during such pretreatment processes. The total concentration of polyphenols in the berry extracts is presented in Table 3.

The total amount of phenolic compounds was greatest in grape berry extracts. There was a noticeable concentration increment of polyphenols in the pulp material after they were subjected to enzymatic treatment. These data indicate that the destruction of polysaccharides during enzymatic maceration promotes the release of some polyphenols. The study found 10 different phenolic compounds in the pulp samples, but only coumarins, quercetin, gallic, and chlorogenic acids were quantified. The qualitative detection data of phenolic compounds in extracts are presented in Table 4.

The largest amount of phenolic derivatives was found in the samples of "Pinot noir" grapes. In the extracts of berries of this variety that underwent prior enzymatic treatment, chlorogenic acid (0.257 g/0.1 kg) and quercetin (0.065 g/0.1 kg) predominated. It is noted that the quercetin content in the extracts of "Pinot noir" and "Aligot" grapes, which underwent enzymatic treatment of the pulp, was higher by 15% and 17%, respectively, compared to untreated samples. In all cases where gallic acid was detected, it has been revealed an essential enlargement in its content after enzymolysis. Strawberry extracts of the "Victoria" variety, prepared from pulp subjected to pectinolysis, showed the highest increase in gallic acid content – by 16%. As shown in Table 4, some samples exhibited the appearance of new peaks after enzymatic hydrolysis. The only exception was the extract of "Victoria" strawberry, where the quercetin peak disappeared after treatment with enzymatic preparations. Among hydroxybenzoic acids, formylsalicylic acid and 4-hydroxybenzoate have been detected. It should be noted that the concentration of 4-hydroxybenzoate sufficiently increased in samples subjected to enzymatic maceration. 4-hydroxybenzoate is related to phenolic compounds associated with plant cell walls. This fact may be the cause of its larger amount in pretreated extracts. The obtained data indicate that the use of enzymatic pulp treatment may contribute to the increased extractability of 4-hydroxybenzoate. By the way, the strict mechanisms of metabolic conversion of 4-hydroxybenzoate in plants remain insufficiently studied.

Table 3. The quantitative content of phenolic compounds in extracts of berries subjected to various methods of preliminary enzymatic maceration.

Culture	Total content of phenolic compounds (mg/100 g)			
	No processing	Pectinase	Cellulase	Pectinase and cellulase
Grapes (variety "Pinot noir")	645.1	657.5	689.4	705.3
Grapes (variety "Aligot")	575.8	596.8	584.6	613.6
Strawberries (variety "Albion")	8.9	9.3	10.2	11.1
Strawberries (variety "Victoria")	9.2	10.1	9.9	10.9
Cherry (variety "Ramon Oliva")	5.8	6.2	5.9	6.5
Cherry (variety "Biraggo Grohl")	3.9	4.2	4.5	5.1

Among the derivatives of hydroxycinnamic acid, two compounds were identified: chlorogenic acid and coumarin. The content of chlorogenic acid in the extract samples showed no essential changes between fermented and native pulp samples. The highest content of chlorogenic acid was found in the extract of “Biraggo Grohl” cherry, which underwent enzymatic treatment. Coumarins were present in significantly smaller amounts in extracts obtained from untreated pulp. However, overall research results suggest that enzymatic treatment of berry pulp contributed to better preservation of phenolic derivatives in pre-treated pulp compared to untreated samples. In the analyzed extracts, the concentration of glucose, fructose, galactose, xylose, arabinose, and mannose was identified and quantitatively measured. The obtained data are presented in Table 5.

As a result of treating the pulp with the enzymatic preparations of pectinase and cellulase, a sufficient enrichment in glucose concentration was detected in all examined samples, directly associated with the breakdown of polysaccharides. Additionally, in most samples, an accretion in the concentration of other simple sugars has been found. This effect was most significant when using a combination of two enzymatic preparations. In some cases, after prior enzymatic maceration, the appearance of peaks of monosaccharides that were absent in the extracts of untreated samples was observed. For example, the presence of 0.1% mannose in the extract of “Pinot noir” grape berries and 0.1% xylose and arabinose in the strawberry samples of the “Victoria” variety. Overall, the obtained data indicate that the increase in the concentration of minor monosaccharides in the samples is associated with the disruption of the cell wall, leading to enhanced extractability of these components.

Discussion

Enzyme preparations can influence oxidases present in samples, leading to ascorbate oxidation. In the study by C. Bender et al. [13], it was found that heating the pulp from blackcurrant berries before pectinolysis resulted in an increase in ascorbate concentration in the juices compared to pectinolysis without heating. However, this effect depended on the type of berries, as it was not observed in raspberries and blueberries. Similarly, the research by M.F. Ramadan and J.T. Moersel [14] showed no reduction in ascorbic acid content in extracts from goldenberry berries subjected to enzymatic treatment. The study by E. Radziejewska-Kubzdela et al. [1] revealed that prolonged treatment of barberry pulp with prior enzymatic and thermal processing caused ascorbate content decrease in the extracts, consistent with the findings of O. Laaksonen et al. [15]. They noted that extracts obtained from enzymatically processed raw material contained significantly less ascorbic acid compared to untreated samples. Furthermore, K.M. Clegg and A.D. Morton [16] suggested that the preservation of ascorbic acid during pretreatment may depend on the specific phenolic compound profile in the raw material. In this study, no significant change in vitamin C content was observed in the samples due to enzymatic treatment. On the contrary, a slight increase in ascorbic acid was detected in extracts treated with a combination of two enzymes.

The whole amount of anthocyanin in the samples of grapes, cherries, and strawberries in this study corresponds to the data obtained by G. Mazza and F.J. Francis [17], A. Chaovanalikit and R.E. Wrolstad [5], and A.S. Stübler et al. [18]. The increase in their concentration in extracts as a result of such treatment may be associated with several mechanisms occurring due to the action of enzymes on berry cell structures. One of the key mechanisms leading to an increase in anthocyanin content is undoubtedly the breakdown of cell structures, particularly cell walls. A. Chaovanalikit and R.E. Wrolstad [5] suggest that enzyme preparations may also contribute to the activation of endoglycosidases, which hydrolyze glycosidic bonds connecting anthocyanins with other molecules, especially sugars. This leads to further release and increased extractability of anthocyanins.

Table 4. Composition of phenolic compounds in berry extracts that underwent enzymatic maceration and in those that were not pre-treated.

	Qu	C-3-r	Qu3-O-g	R	4-h	Fsa	P3-O-g	Ga	Cha	C
GPN	n/e	-	e	n/e	n/e	-	-	n/e	n/e	n/e
GA	n/e	e	e	n/e	n/e	-	-	-	n/e	n/e
SA	n/e	n/e	n/e	n/e	-	n/e	-	e	n/e	n/e
SV	n	n/e	n/e	n/e	n/e	n/e	e	n/e	n/e	n/e
CRO	e	-	e	n/e	n/e	-	-	e	n/e	-
CBG	e	-	-	n/e	-	-	-	-	n/e	-

Note: GPN – grape variety “Pinot noir”; GA – grape variety “Aligot”; SA – strawberry variety “Albion”; SV – strawberry variety “Victoria”; CRO – cherry variety “Ramon Oliva”; CBG – cherry variety “Biraggo Grohl”; t/u – detected in both processed and unprocessed samples; e – found only in samples treated with one of the enzymes or a mixture of them; n – found only in untreated samples; Qu – Quartzetin; C-3-r – Cyanidin-3-rutinoside; Qu3-O-g – Quercetin 3-O-glucoside; R – Rutin; 4-h – 4-hydroxybenzoate; Fsa – Formyl salicylic acid; P3-O-g – Peonidin 3-O-glucoside; Ga – Gallic acid; Cha – Chlorogenic acid; C — Coumarin.

In addition, according to E. Radziejewska-Kubzdela et al. [1], the activity of pectinolytic enzymes may affect the activity of polyphenol oxidase, which oxidizes anthocyanins.

Pretreatment with enzyme preparations may help prevent the oxidation process, preserving the anthocyanins to remain intact. The impact of enzymatic processing and high-temperature incubation on anthocyanin yield has been observed in several other studies. N. Alberici et al. [19] investigated anthocyanin yield in blackcurrant and blueberry samples afterward enzymolysis of the mash. They found that enzymatic treatment led to a higher anthocyanin yield compared to untreated samples, indicating improved release of anthocyanins from the fruit material. Similarly, G. Ljod et al. [20] found the influence of temperature and enzymatic treatment on anthocyanin yield in berry pulp. They noticed that both treatments increased the anthocyanin yield in the obtained samples compared to untreated samples. The results suggest that enzymatic treatment may enhance the extraction and release of anthocyanins from berry material.

One of the main mechanisms for increasing the concentration of polyphenols is likely associated with the breakdown of the cell wall using pectinase and cellulase. Enzymatic pre-treatment contributes to the release of polyphenols, facilitating their extraction and increasing their content in the extract. Additionally, according to the study by A. Nicolescu et al. [21], the introduction of enzymes can activate internal enzymatic systems, such as polyphenol oxidases present in berries. These enzymes accelerate the oxidative processes of polyphenols, leading to the formation of polymeric and oxidized forms. Furthermore, enzymes can contribute to the hydrolysis of glycosidic bonds connecting polyphenols with other molecules, such as sugars or organic acids. This biochemical process can cause the damage of complexes with polyphenols and increase the availability of the ones for extraction. N. Costoya et al. [22] studied the use of various enzymes (Cellubrix, Neutrase, and Viscozyme) on the grape pulp of Garnatxa (white) and Cabernet (red) sorts to increase the quantity of antioxidant compounds during extraction. Enzymatic treatment, especially when using a mixture of three enzymes, significantly increased the phenol extraction by 21% for Garnatxa and 46% for Cabernet compared to untreated samples. Additionally, enzymatic treatment also contributed to the increased solubility of several compounds in water. This suggests that enzymatic pre-treatment can improve the solubility of compounds, potentially enhancing their bioavailability and extraction efficiency.

It was found that the pre-treatment of berries with enzyme preparations promotes more efficient extraction of polyphenols and increases their concentration in the extract. These results are consistent with previous studies that also reported similar effects. For example, the study by L.N. Lieu and V.V.M. Le [23], comparing enzymatic treatment and ultrasound treatment of grape must, showed an enlargement in the concentration of phenolic derivatives in berry extracts after enzymatic treatment. However, the study conducted by E. Radziejewska-Kubzdela et al. [1] did not find such a dependency. Instead, they noted a reduction in the total polyphenol content in berry extracts after pre-enzymatic treatment. These results may be related to the accidental activity of esterase present in the used enzyme or damage of polyphenolic molecules in processing conditions. Moreover, residual polyphenol oxidases present in grape pulp may also contribute to the reduction of phenolic and antioxidant contents. The mentioned researchers also demonstrated that the use of enzymatic or thermal pre-treatment contributes to the increased extractability of 4-hydroxybenzoate compared to ultrasound treatment or untreated samples. The specific mechanisms underlying this improvement may vary and include increased enzymatic activity, breakdown of cell walls, or enhanced solubility of 4-hydroxybenzoate during pre-enzymatic treatment processes.

Within the study, it was identified that pre-treatment of berries with pectinase and cellulase enzyme preparations contributes to an increase in the monosaccharide content in their extracts. One of the main mechanisms causing the elevation of monosaccharide concentration is associated with the breakdown of polysaccharides in cell walls. Pectinolytic enzyme pre-treatment promotes the breakdown of these polysaccharides into monosaccharides, primarily glucose and fructose. According to the study by K.R. Corbin et al. [24], this process also facilitates access to monosaccharides for extraction, leading to their increased concentration in the extract. Scientists argue that pectinolytic enzymes can activate glycosidases, which hydrolyze glycosidic bonds connecting carbohydrates with glycoproteins, also contributing to the release of monosaccharides, and increasing their concentration in the extract. Additionally, they suggest that enzymatic pre-treatment may stimulate internal amylases, which break down oligosaccharides into monosaccharides. The researchers also found a reduction in the concentration of dietary fibers and an increment of the amount of free simple sugars after enzymatic hydrolysis of fruit peel, consistent with the breakdown of complex polysaccharides into simpler sugars [25, 26].

This study also demonstrates that after pre-treatment of berries with pectinase and cellulase enzyme preparations, there is an increase in the concentration of monosaccharides in the extracts, confirming the consistency of the obtained results with data presented in the works of other researchers.

Conclusions

This study investigated the impact of pre-processing berry pulp with the enzymatic agents pectinase and cellulase on the content of bioactive substances in their extracts. It was found that enzymatic

Table 5. Sugar content in berry extracts that were pre-treated with different methods of enzymatic maceration.

Culture	Processing method	Content (g/100g)					
		Glucose	Fructose	Galactose	Xylose	Arabinose	Mannose
Grapes (variety "Pinot noir")	No processing	4.2	3.8	0.7	0.8	0.6	0
	Pectinase	23.2	4.1	0.8	0.7	0.6	0
	Cellulase	12.5	7.8	1.1	1.2	0.9	0.1
	Pectinase and cellulase	45.5	7.7	1.3	1.1	1	0.1
Grapes (variety "Aligot")	No processing	5.7	4.6	0.5	0.3	0.4	0.1
	Pectinase	26.7	4.5	0.6	0.4	0.3	0.1
	Cellulase	14.3	8.7	0.7	0.7	0.4	0.1
	Pectinase and cellulase	56.5	9.1	0.6	0.7	0.5	0.2
Strawberries (variety "Albion")	No processing	7.4	8.5	0.2	0	0	0
	Pectinase	34.8	8.7	0.3	0	0	0
	Cellulase	16.7	9.4	0.3	0	0	0
	Pectinase and cellulase	57.8	9.4	0.3	0	0	0
Strawberries (variety "Victoria")	No processing	6.5	7.3	0.1	0	0	0
	Pectinase	27.8	8.1	0.1	0	0	0
	Cellulase	14.3	9.2	0.2	0	0	0
	Pectinase and cellulase	48.9	9.7	0.2	0.1	0.1	0
Cherry (variety "Ramon Oliva")	No processing	6.8	7.6	0.2	0.4	0.2	0.2
	Pectinase	29.9	8.2	0.5	0.5	0.2	0.2
	Cellulase	13.5	8.4	0.4	0.5	0.3	0.2
	Pectinase and cellulase	57.4	8.3	0.5	0.6	0.4	0.2
Cherry (variety "Biraggo Grohl")	No processing	9.1	5.4	0.5	0.6	0.3	0.1
	Pectinase	39.4	5.2	0.7	0.7	0.4	0
	Cellulase	17.1	6.3	0.8	0.7	0.3	0.1
	Pectinase and cellulase	68.6	6.4	0.8	0.7	0.5	0.1

maceration led to a slight increase in ascorbic acid concentration in extracts from all examined berries. The largest amount of vitamin C has been revealed in strawberry samples of the "Victoria" variety treated with both enzymatic agents. There was also a substantial increment of the overall polyphenols and anthocyanins content in the examined extracts. The "Pinot noir" grape variety demonstrated the highest increase in anthocyanin content (140%) when using a combination of both enzymatic agents. Selective identification and quantitative measurement of certain anthocyanins in the "Pinot noir" grape variety revealed that delphinidin-3-monoglucoside and malvidin-3-monoglucoside were the predominant anthocyanins present. Enzymatic processing of the grape pulp of this variety resulted in a 20% increase in the malvidin-3-monoglucoside concentration in enzymatic-treated pulp, instead of native material. The content of 3-monoglucoside-acetate in extracts increased by 2.5 times after treatment with both agents.

Ten phenolic derivatives have been detected in the analyzed extracts. The "Pinot noir" grape variety exhibited the largest number of phenolic molecules, compared to the other samples. Extracts from its berries, pre-treated with enzymatic agents, contained a predominant amount of chlorogenic acid (0.257 g/0.1 kg) and quercetin (0.065 g/0.1 kg). The quercetin content in extracts from "Pinot noir" and "Aligot" grape varieties, prepared from pectinolyzed pulp, was 15% and 17% higher, respectively, in comparison to native material. Additionally, an essential augmentation in gallic acid content after enzymatic processing was observed in all samples where it was detected. Strawberry extracts of the "Victoria" variety showed the most significant increase in gallic acid content (16%). In all analyzed extracts, a substantial increase in glucose concentration was observed, directly associated with polysaccharide

breakdown, as well as a less pronounced increase in other monosaccharides. The use of a combination of two enzymatic agents showed the highest efficiency in increasing monosaccharide concentration.

The study confirmed that the application of pectinase and cellulase positively influences the content of bioactive substances in extracts from grapes, cherries, and strawberries. No significant differences in efficacy between these two agents were detected. In most cases, the use of a combination of both agents resulted in the most positive outcome. These findings suggest the potential of enzymatic maceration of berries for further appliance in the food industry. However, for a more in-depth understanding of the mechanisms underlying such effects on various compound classes, additional research is required.

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