

Article

Influence of Enzymatic Hydrolysis on Composition and Technological Properties of Black Currant (*Ribes nigrum*) Pomace

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Abstract: Blackcurrant (*Ribes nigrum*) is valued for its health-promoting compounds, many of which remain in the pomace after juice extraction. Berry pomace can be considered a valuable source of dietary fiber. However, it is typically dominated by insoluble dietary fiber (IDF), and the soluble-to-insoluble fiber ratio is often nutritionally suboptimal. The aim of this study was to evaluate the influence of enzymatic hydrolysis on the composition and technological properties of blackcurrant pomace (BCP). Three commercial enzyme preparations—Viscozyme[®] L, Celluclast[®] 1.5 L, and Pectinex[®] Ultra Tropical (Novozymes A/S, Denmark)—were used for enzymatic hydrolysis, which was conducted at 50 °C for 1 h. The enzymatic treatments altered BCP's chemical composition and technological properties. Pectinex[®] Ultra Tropical and Viscozyme[®] L primarily hydrolyzed SDF, while Celluclast[®] 1.5 L was more effective on IDF, resulting in increased SDF content and an improved SDF/IDF ratio. Enzymatic hydrolysis reduced the oil retention capacity and impaired stabilizing properties, but it increased both the water retention capacity and the solubility index. It was found that the creaming index of the pomace deteriorated with decreased IDF content. The findings indicate that the effects of enzymatic modification on BCP's composition and technological properties can vary significantly, supporting its potential application in the development of novel food products.

Keywords: black currant pomace; enzymatic hydrolysis; dietary fiber; technological properties



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1. Introduction

Today, health-conscious consumers are seeking healthier foods with higher nutritional value. In 2020, the European Commission introduced the Farm to Fork strategy to establish a fair, reliable, and environmentally friendly food system. This strategy aims to reduce the climate impact of the European Union food system. The growing emphasis on sustainable food systems and the circular economy has increased attention to the recovery and reuse of agro-industrial byproducts. Pomace valorization directly supports this strategy by converting byproducts from berry, fruit, and vegetable processing, such as pomace, into valuable ingredients for food, feed, or nutraceutical applications. This approach reduces food waste and environmental impact, promoting the efficient use of agricultural resources and aligning with key objectives of the “Farm to Fork” strategy. In the fruit processing industry, pomace—the solid residue left after juice extraction—is a valuable yet underexploited source of nutrients and functional components [1]. Black currant (*Ribes nigrum*), a woody shrub of the *Grossulariaceae* family, produces berries rich in pigments, vitamins,

organic acids, and polyphenols [2]. During juice production, many of these beneficial compounds remain in the pomace, primarily consisting of berry skins and seeds [3].

Black currant pomace (BCP) is especially rich in dietary fiber, predominantly in its insoluble form [4]. While insoluble dietary fiber (IDF) contributes to digestive health, it offers limited technological functionality and different physiological benefits than soluble dietary fiber (SDF). SDF, on the other hand, has been widely recognized for its role in regulating blood glucose and cholesterol levels, supporting the gut microbiota, and improving the textural properties of food products [5]. According to recommendations from the World Health Organisation (WHO) and the European Food Safety Authority (EFSA), the recommended daily intake of dietary fiber is 25 g per person. However, actual consumption often falls significantly below this target [6,7]. Dietary fiber's health benefits and technological functionality are primarily influenced by the soluble-to-insoluble fiber ratio, with an optimal recommended ratio of 1:3 [8]. However, in most cases, this ratio is significantly higher in pomace, which complicates its utilization from a technological standpoint. Therefore, researchers are exploring strategies to reduce IDF content and increase the proportion of SDF [9–11]. Elevated levels of IDF in food products can negatively affect texture and color, depending on its composition, properties, and concentration [12]. One of the most widely used methods for modifying dietary fiber is enzymatic treatment, which alters the product's composition, functional characteristics, and technological properties.

Consequently, strategies that increase the proportion of SDF in fiber-rich residues are gaining interest in the development of functional ingredients [1,13]. Enzymatic hydrolysis represents one strategy, offering a targeted and mild approach to modifying plant-derived materials. Enzymes such as cellulase, hemicellulase, and pectinase can partially break down complex polysaccharides in the plant cell wall, converting insoluble fiber into soluble forms and altering the material's functional properties [14]. While the benefits of enzymatic modification have been demonstrated in various fruit and grain by-products [15], limited research has focused specifically on the impact of this treatment on BCP. Moreover, limited research has focused on the technological properties of BCP.

This study was designed to evaluate the influence of commercially available enzymes (Viscozyme[®] L, Pectinex[®] Ultra Tropical, and Celluclast[®] 1.5 L) on the chemical composition and technological properties (water and oil retention capacities, water solubility index, bulk density, stability of emulsions depending on pH, color changes) of BCP. Viscozyme[®] L and Pectinex[®] Ultra Tropical contain pectinolytic and cellulolytic activity, while Celluclast[®] 1.5 L contains mainly cellulolytic activity. The hypothesis of the research is that enzymatic hydrolysis of BCP using specific carbohydrases (e.g., cellulase, hemicellulase, and pectinase) will decrease the content of IDF while increasing the proportion of SDF, thereby improving the functional and technological properties of the BCP, such as water and oil retention capacity and emulsion stability. The outcomes are expected to support the development of value-added ingredients from black currant berry processing waste, with potential applications in health-oriented and clean-label food products.

2. Materials and Methods

2.1. Black Currants Pomace Characteristics

Fresh BCP was generously provided by JSC 'Juodoji uoga' (Biržai, Lithuania). Following juice extraction, the remaining BCP was immediately subjected to drying at 35–40 °C for 48 h until it reached a moisture level of approximately $7.97 \pm 0.10\%$. The dried material was then ground using a ZM 200 mill (Retsch, Haan, Germany) to obtain particles below 0.5 mm.

2.2. Enzymatic Treatment of Black Currants Pomace

For enzymatic modification, commercial enzymes supplied by Novozymes A/S (Bagsvaerd, Denmark) were used—specifically Viscozyme[®] L (100 FBG/g composed of β -glucanases, pectinases, hemicellulases, xylanases), Pectinex[®] Ultra Tropical (5000 PECTU/g, composed of pectinases, cellulases, hemicellulases, β -glucanases), and Celluclast[®] 1.5 L (700 EGU/g, composed of cellulases). In each treatment, 37.5 mL of distilled water was combined with 2.5 g of BCP and 250 μ L of a commercial enzymatic solution. The concentration of commercial enzymes was chosen based on the manufacturer's recommendations and literature source [16,17]. Control samples were prepared using distilled water only, without the addition of enzymes. Hydrolysis was conducted at 50 °C for one hour in a shaking water bath set to 200 rpm (under initial pH without further adjustment). The mixtures were then heated at 90 °C for 20 min to terminate enzymatic activity and allowed to cool to room temperature (approximately 20 °C).

For sugar composition analysis, the hydrolyzed mixtures were centrifuged at 8000 rpm for 20 min using a Microcen 23 centrifuge (Ortoalresa, Madrid, Spain), after which the supernatant was freeze-dried (Harvest Right, North Salt Lake, UT, USA). For the analysis of SDF and IDF and functional property assessments, the entire hydrolyzed mixtures were freeze-dried without separation. All freeze-dried BCP samples were stored at 4 °C in airtight containers to maintain their integrity until further testing. The schema of the experiment is given in Figure 1.

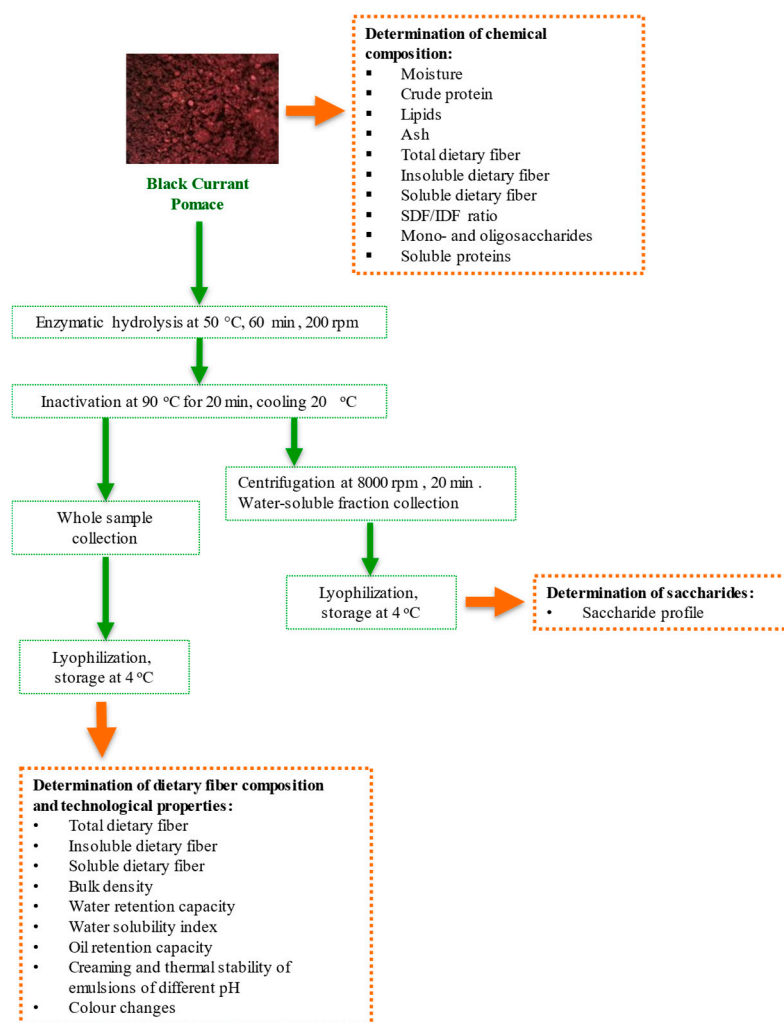


Figure 1. Experimental scheme.

2.3. Chemical Composition Determination

Moisture content was assessed by drying 1 g of the sample at 105 °C until a constant weight was achieved, following the procedure outlined in AOAC Method 925.10-1925 [18]. Lipid content was determined via Soxhlet extraction using hexane for 3 h with 3 g of sample, following AOAC Method 948.22 [18]. Crude protein concentration was measured using the Kjeldahl technique (conversion factor $N \times 6.25$) on 1 g of sample, based on AOAC Method 978.04 [18]. To quantify the ash content, 2 g of the sample were initially charred for 30 min, followed by incineration in a muffle furnace at 625 °C for 2 h, in accordance with AOAC Method 930.05 [18]. Total dietary fiber (TDF), including its soluble (SDF) and insoluble (IDF) fractions, was analyzed using the Total Dietary Fiber Assay Kit provided by Megazyme International (Wicklow, Ireland), according to the manufacturer's protocol based on AACC Method 32-07.01 and 991.43 [19]. In brief, 1 g of sample was suspended in 40 mL of 0.05 M MES-Tris buffer (pH 8.2) and subjected to enzymatic digestion. The sample was first treated with α -amylase (50 μ L, 95 °C, 30 min), followed by protease (100 μ L, 60 °C, 30 min), under continuous agitation at 120 rpm. The pH of the mixture was then adjusted to a range of 4.1–4.8 using 1 N HCl (0.56 mL), after which amyloglucosidase (200 μ L) was loaded and the hydrolysis continued (60 °C, 30 min) under the same stirring conditions. Following enzymatic treatment, the mixture was filtered using a Fibertec 1023 E system (Foss System, Hilleroed, Denmark). The solid residue was sequentially rinsed with hot distilled water (10 mL, 70 °C), then with 95% ethanol (10 mL) and acetone (10 mL), followed by drying at 103 °C overnight. The resulting dry mass was recorded as the IDF fraction. The filtrate and washings were combined and mixed with four volumes of 95% ethanol for SDF quantification. After standing for one hour at ambient temperature, the mixture was filtered. The collected residue was washed with 78% and 95% ethanol, followed by acetone, then dried and weighed. The protein and ash contents in both the IDF and SDF fractions were determined and subtracted to yield the fibre values. TDF was calculated as the sum of SDF and IDF.

2.4. Analysis of Saccharides

To analyze saccharide content, 10 mg of the freeze-dried sample were reconstituted in 1 mL water (Millipore-grade). The identification of mono- and disaccharides followed the method outlined by Bytautaitė et al. [20], utilizing a HPLC system of Thermo Scientific Ultimate 3000 equipped with the refractive index detector RefractoMax 521 (Thermo Fisher Scientific, Waltham, MA, USA). Separation of saccharide constituents was carried out with a tandem setup of SUGAR KS-802 and KS-801 columns (8.0 mm ID \times 300 mm each; Shodex, Tokyo, Japan), with ultrapure water serving as a mobile phase. The column temperature was 80 °C, and the system operated under isocratic conditions at a flow speed of 0.5 mL/min. An amount of 10 μ L of the sample was injected, and the total run time was 60 min. Data acquisition and chromatogram processing were conducted with the software Chromeleon 7 (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. The Evaluation of Technological Properties

BCP's water retention capacity (WRC) was assessed following the procedure described by Yu et al. [21], applying minor adjustments. In a centrifuge tube, 0.2 g of the tested sample was added and combined with 6 mL of distilled water, covered, stored at 21 °C for 18 h, then centrifugated at 5000 rpm for 20 min. After centrifugation, the liquid was decanted, while the remaining material was scaled. The sample was dried at 105 °C to a uniform mass and reweighed. WRC was calculated based on Equation (1).

$$\text{WRC (g/g)} = (M1 - M2)/M2 \quad (1)$$

where M1 is the weight of residues before drying (g); M2 is the weight of residues after drying (g).

The supernatant obtained after decantation was used for water solubility index (WSI) assessment. It was dried at 105 ± 2 °C up to a constant mass. The water solubility index (WSI) was then determined using Equation (2):

$$\text{WSI (\%)} = (M/M_0) \times 100 \quad (2)$$

where M is the weight of the dried soluble material (g); M0 is the dry pomace weight used for dry matter analysis (g).

The oil retention capacity (ORC) of BCP was determined based on the method of Yu et al. [21], with slight modifications. A portion of the sample (0.2 g) was combined with 2 g of sunflower oil and allowed to stand at 21 °C for 1 h, then subjected to centrifugation at 3000 rpm for 10 min. After centrifugation, the unabsorbed oil was decanted, and the weight of the remaining pellet was recorded. ORC was calculated using Equation (3).

$$\text{ORC (g/g)} = (W_1 - W_0)/W_0 \quad (3)$$

where W1 is the mass of the pellets obtained after centrifugation (g); W0 is the mass of the dry sample, recalculated in dry matter (g).

BCP's bulk density (BD) was measured following the procedure described by Jagelaviciūtė et al. [22]. BCP (0.2 g) was accurately scaled into a graduated test tube. The tube was then gently tapped against a flat surface 20 times to allow for sample settling, after which the volume occupied by the material was recorded. BD was calculated using Equation (4).

$$\text{BD (g/mL)} = M/V \quad (4)$$

where M is the mass of the sample, recalculated in dry matter (g); V is the volume of sample in the test tube (mL).

2.6. Evaluation of Emulsion Stability

To prepare emulsions, 0.16 g of BCP was combined with 8 mL of distilled H₂O and 8 g of sunflower oil (Natura, Argentina, San Lorenzo) in graduated test tubes. The mixture was homogenized using a high-speed homogenizer (IKA® T-25 digital, Ultra-Turrax, Staufen, Germany) operating at 10,000 rpm for 5 min. The stability of the prepared emulsions was assessed under both static storage conditions (static stability) and thermal treatment conditions (thermal stability) following the method described by Keršienė et al. [23]. Thermal treatment was performed by incubating the emulsions in a water bath at 80 °C for 30 min. Emulsion stability was evaluated based on the volume of the separated serum (VS) in relation to the total volume of the sample (VB) following 30 min of storage at room temperature, or after thermal treatment, as well as after 1, 7, and 14 days of storage at refrigeration temperature. The degree of emulsion phase separation (creaming index, %) was calculated using Equation (5). The effect of pH on emulsion stability was investigated by replacing distilled water with buffer solutions prepared from 0.1 M citric acid and 0.2 M disodium hydrogen phosphate (Na₂HPO₄) to adjust the final pH to 4.0, 6.0, and 8.0.

$$\text{Creaming index (\%)} = (SV/TV) \times 100 \quad (5)$$

where SV is the volume of serum released (mL); TV is the total emulsion volume (mL).

2.7. Color Analysis

Color characteristics were determined with a colorimeter (Konica Minolta, Tokyo, Japan) operating in the $L^*a^*b^*$ color space. The numerical value of L^* describes lightness from 0 to 100 (0 is black, 100 is white), a^* value indicates green-red color, and b^* value indicates blue-yellow color. The BCP sample was placed in a plate and evenly distributed over the entire area of the plate. Then, three zones from the bottom of the plate were randomly measured. For color comparison, the color difference (ΔE) was calculated according to Sivam et al. [24]. Chroma and ΔE values were calculated according to Equations (6) and (7):

$$\text{Chroma} = \sqrt{a^2 + b^2} \quad (6)$$

$$\Delta E = \sqrt{[(L^*0 - L^*)^2 + (a^*0 - a^*)^2 + (b^*0 - b^*)^2]} \quad (7)$$

where L^*0 , a^*0 , and b^*0 are the values of the control sample.

2.8. Determination of Water-Soluble Protein Content

The concentration of water-soluble proteins was quantified using the Bradford assay, a colorimetric method that involves binding proteins to Coomassie Brilliant Blue G-250 dye [25]. Bradford reagent was prepared by dissolving 0.1 g Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol and mixing it with 100 mL of 85% phosphoric acid. The obtained solution was then diluted with distilled water to a final volume of 1 L. A 0.16 g portion of the BCP was weighed into a 50 mL polypropylene centrifuge tube and extracted with 8 mL of distilled water. The mixture was homogenized for 1 min at 1000 rpm, followed by centrifugation at 6000 rpm for 15 min. The resulting supernatant was filtered through a membrane filter to remove particulates. An aliquot of 0.1 mL of the filtered supernatant was mixed with 3.0 mL of Bradford reagent and incubated at room temperature for 10 min. Absorbance was measured with a UV-Vis spectrophotometer at 595 nm. Protein concentration was determined from a standard calibration curve generated with known concentrations of bovine serum albumin. The final protein content in the sample was calculated using Equation (8):

$$\text{Soluble proteins (mg/g)} = m_1/m_2 \quad (8)$$

where m_1 —protein concentration determined from the calibration curve, mg/mL; m_2 —mass of the sample in the extract, g/mL.

2.9. Statistical Analyses

All experimental measurements were conducted in triplicate. Data were expressed as mean values accompanied by standard deviations, calculated using Microsoft Excel 2019 (Microsoft Corp., Albuquerque, NM, USA). Statistical analyses were conducted with the Statgraphics Centurion 19 software. One-way analysis of variance (ANOVA) was applied to assess differences among sample groups, and Tukey's honest significant difference (HSD) post hoc test was used to identify statistically significant differences at a confidence level of $p \leq 0.05$.

3. Results and Discussion

3.1. Chemical Composition of BCP

The chemical composition of dried and crushed BCP is presented in Table 1. TDF followed by lipids and protein are the main components of BCP. The SDF/IDF ratio influences DF's physiological and technological properties and is important from a nutritional perspective. The SDF/IDF ratio in BCP is 1:5, which is lower than the recommended range of

1:2– [8,26]. Therefore, it is appropriate to explore additional technological modifications to enhance the SDF content. The difference in SDF and IDF hydration properties significantly influences the technological properties of pomace and the capabilities of food products [27]. The ratio between SDF and IDF also significantly affects physiological functions including gut motility and microbiota diversity, thereby influencing both health outcomes and functional properties in food applications [28].

Table 1. Chemical composition of BCP.

Parameter	Content
Moisture, g/100 g	7.97 ± 0.10
Crude protein, g/100 g d.w.	9.05 ± 0.33
Soluble proteins, g/100 g d.w.	5.09 ± 0.01
Lipids, g/100 g d.w.	13.85 ± 0.27
Ash, g/100 g d.w.	3.82 ± 0.02
TDF, g/100 g d.w.	49.24 ± 0.82
IDF, g/100 g d.w.	40.95 ± 0.78
SDF, g/100 g d.w.	8.29 ± 0.17
SDF/IDF ratio	1:5
Mono-/oligosaccharides, g/100 g d.w. *	24.04 ± 0.64

* Mono- and oligosaccharides = 100 – (crude protein + lipids + ash + TDF). Data values are expressed as means with the standard deviation ($n = 3$).

Reißner et al. [29] reported a different chemical composition of BCP from Germany with higher content of lipids (20.21 g/100 g d.w.), protein (15.71 g/100 g d.w.), and IDF (51.16 g/100 g d.w.) and lower content of SDF (3.97 g/100 g d.w.) and ash (2.66 g/100 g d.w.). In comparison, Alba et al. [8] evaluated the chemical composition of BCP from the UK and Poland, reporting higher contents of IDF (61.3 and 61.9 g/100 g d.w., respectively) and SDF (25.1 and 30.0 g/100 g d.w., respectively). The chemical composition of pomace may vary due to the characteristics of the berry variety, climatic conditions, soil, and other environmental factors during cultivation, as well as the processing technology used. The amount and composition of dietary fiber in pomace may vary depending on the type of juice production technology used. In industrial juice production, enzymatic treatment with pectinases [30] is most often used before pressing, which, by hydrolyzing pectin, reduces the viscosity of the juice and increases the efficiency of the pressing process and the yield of juice. The insufficient enzymatic treatment has an impact on the pomace's dietary fiber composition, and pomaces may contain more pectin and a higher content of SDF, as well as a higher content of mono- and oligosaccharides [29]. The high levels of SDF and mono- and oligosaccharides in the studied BCP indicate that the black currant berry was untreated or insufficiently treated with enzymes before juice pressing.

3.2. DF Composition of Enzymatically Treated BCP

The modification of pomace by enzymatic hydrolysis is often used to increase the content of SDF and oligosaccharides. Since the polysaccharides of blackcurrant berry are dominated by pectin (11–16% d.w.), hemicelluloses (14–15% d.w.), and cellulose (14–17% d. m.) [8], different carbohydrases were selected for hydrolysis.

The pectinolytic enzymes—Pectinex® Ultra Tropical and Viscozyme® L—effectively hydrolyzed SDF (Table 2). Meanwhile, IDF was hydrolyzed only 17–19%, respectively. The higher degree of SDF hydrolysis had a negative impact on the SDF/IDF ratio. In contrast, Celluclast 1.5 L (cellulolytic enzymes) hydrolyzed mainly IDF and increased the SDF content by 7.11%. The SDF/IDF ratio in BCP hydrolyzed with Celluclast® 1.5 L was also improved. Sezer et al. [31] found similar trends after cherry pomace hydrolysis using cellulase (600 units/g of pomace). There is a lack of information in the literature on the enzymatic modification or other pretreatments of BCP to increase the amount of

SDF, while other berries are more studied. Šimkutė et al. [32] reported that cellulolytic enzyme preparation (Celluclast 1.5 L) increased the content of SDF by 13% and enhanced the ratio of IDF/SDF in lingonberry pomace from 7.6 to 6.1; however, the obtained ratio did not achieve the recommended value. In cranberry pomace, Celluclast[®] 1.5 L increased SDF content by 5.6% [14]. Cereals, legumes, and fruit byproducts processing opportunities for increasing SDF content are more studied, applying not only enzymatic treatments but also physical treatments, such as thermal and extrusion techniques, as well as hydrostatic pressures [15]. The application of high hydrostatic pressure has been shown to significantly enhance the SDF content in fruit byproducts, with increases of approximately 15% in mango peel and 95% in orange peel. Similarly, extrusion processing markedly elevated SDF levels in garlic skin and wheat bran by 199% and 98%, respectively [15]. Enzymatic treatment has also proven effective; for instance, Gu et al. [33] demonstrated that using Viscozyme L significantly improved the SDF extraction yield from tomato peels, reaching up to 72%. In another study, Mrabet et al. [34] observed a threefold increase in SDF content in date fruit following Viscozyme[®] L treatment under optimized conditions. Additionally, Yoon et al. [35] utilized a cellulase-rich enzyme extract derived from edible snails, which successfully enhanced carrot pomace's alcohol-soluble dietary fiber content. Other studies have shown that apple pomace hydrolysis, using cellulolytic enzymes, improves SDF by 20% [22]. In another study, hydrolysis using cellulase yields the highest SDF content (18.7%) in apple pomace [36]. Xiao et al. [37] reported that enzymatic-ultrasound treatment can significantly increase its SDF/IDF ratio, up to 17.07% in sea buckthorn pomace. The enzymatic treatment of pineapple pomace with cellulase significantly increased the SDF content and SDF/TDF ratio, which improved from 2.2 to 9.5% [10]. Enzymatic treatments applying Pectinex[®] Ultra SP-L, Viscozyme[®] L, and Celluclast[®] 1.5 L significantly increased the SDF content in carob, artichoke, apple, and broccoli byproducts [9].

Table 2. Influence of commercial enzymes on the amount and composition of BCP fiber.

	Control	Viscozyme [®] L	Pectinex [®] Ultra Tropical	Celluclast [®] 1.5 L
TDF, g/100 g d.w.	49.24 ± 0.82 ^c	37.22 ± 0.74 ^a	36.46 ± 0.33 ^a	40.06 ± 0.90 ^b
Decrease of TDF, %	-	24.42 ± 1.45 ^b	25.95 ± 0.64 ^b	18.64 ± 1.76 ^a
SDF, g/100 g d.w.	8.29 ± 0.17 ^b	3.33 ± 0.09 ^a	3.29 ± 0.02 ^a	8.88 ± 0.42 ^b
Decrease of SDF, %	-	59.88 ± 1.11 ^a	60.30 ± 0.22 ^a	-
IDF, g/100 g d.w.	40.95 ± 0.78 ^c	33.89 ± 0.65 ^b	33.17 ± 0.31 ^b	31.18 ± 0.48 ^a
Decrease of IDF, %	-	17.24 ± 1.58 ^a	18.99 ± 0.76 ^a	23.86 ± 1.18 ^b
SDF/IDF ratio	0.202	0.099	0.099	0.255

Different lowercase letters a–c in row show that values are significantly different ($p < 0.05$). Decrease in dietary fiber calculated based on control.

The analysis was also performed using HPLC to assess the effect of hydrolysis on changes in mono- and disaccharide contents. The study's results are presented in Figure 2. During the study, galacturonic acid, glucose, fructose, and sucrose, as well as traces of sugar alcohols, were identified in BCP. The enzymatic treatment led to higher contents of mono- and disaccharides. The enzymatic treatment of BCP using Viscozyme[®] L, Pectinex[®] Ultra Tropical, and Celluclast[®] 1.5 L increased the amount of galacturonic acid (by 58.4, 47.7, and 35.0%, respectively), which is the major constituent in pectin. Using enzymes Viscozyme[®] L, Pectinex[®] Ultra Tropical, and Celluclast[®] 1.5 L, the sucrose content was increased by 8.5, 74.6, and 64.4% compared with unhydrolyzed samples, respectively, while fructose content increased up to 100, 51.9 and 98%, respectively.

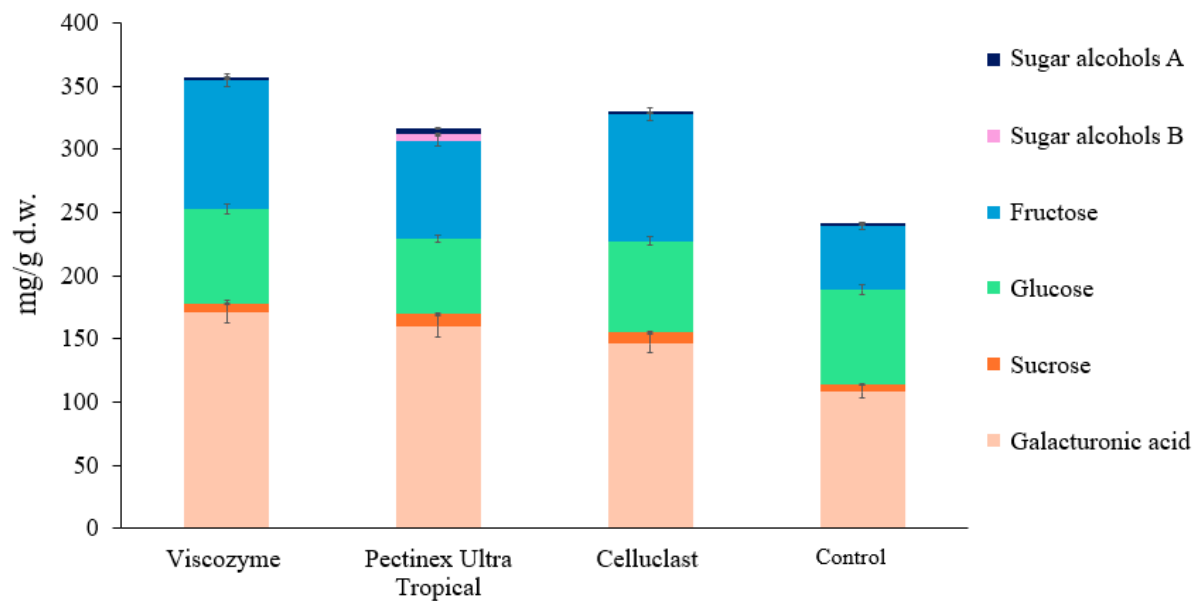




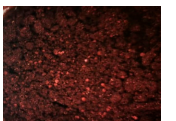

Figure 2. Effect of enzymatic hydrolysis on the saccharide profile of BCP.

3.3. Technological Properties of Modified BCP

The enzymatic hydrolysis of pomace and dietary fiber changes not only have an impact on the SDF/IDF ratio but also influence technological properties. The modification breaks down the glycosidic bonds of high molecular-weight carbohydrates, thereby exposing more groups and affecting the technological properties of pomace [38].

The results of the color and technological properties (oil retention capacity, water retention capacity, water solubility index, and bulk density) of the hydrolyzed BCP are presented in Table 3.

Table 3. Technological properties and color changes of BCP after enzymatic modification.

	Control	Viscozyme® L	Pectinex® Ultra Tropical	Celluclast® 1.5 L
				
L*	25.05 ± 0.39 ^b	24.38 ± 0.35 ^b	21.36 ± 0.64 ^a	24.79 ± 0.21 ^b
a*	19.26 ± 0.22 ^b	20.19 ± 0.28 ^c	15.94 ± 0.32 ^a	21.08 ± 0.08 ^d
b*	4.04 ± 0.05 ^b	4.61 ± 0.11 ^d	3.08 ± 0.04 ^a	4.30 ± 0.05 ^c
ΔE	-	1.28 ± 0.09 ^a	5.06 ± 0.27 ^b	1.87 ± 0.13 ^a
Chroma	19.68 ± 0.22 ^b	20.71 ± 0.30 ^c	16.24 ± 0.32 ^a	21.51 ± 0.08 ^d
ORC, g/g d.w.	3.5 ± 0.1 ^d	2.2 ± 0.1 ^a	2.5 ± 0.2 ^{ab}	3.2 ± 0.1 ^{cd}
WRC, g/g d.w.	5.0 ± 0.2 ^a	6.8 ± 0.2 ^c	6.2 ± 0.1 ^b	5.6 ± 0.3 ^a
WSI, %	32.3 ± 1.2 ^a	37.6 ± 1.7 ^b	48.1 ± 0.1 ^c	37.7 ± 2.1 ^b
Bulk density, g/mL	0.17 ± 0.01 ^a	0.16 ± 0.01 ^a	0.18 ± 0.00 ^a	0.17 ± 0.01 ^a

Mean ± standard deviation values in row with different lowercase letters are significantly different ($p < 0.05$).

Enzymatic treatment had an impact on the color characteristics of pomace. Hydrolysis with Viscozyme® L and Celluclast® 1.5 L increased the values of a* and b*. Szymanowska et al. [39] found that during the pressing of berry juice, using pectinases and cellulases, quantitative and qualitative changes in phenolic compounds occur between them and anthocyanins, and the addition of enzymes increased the amount of anthocyanins in the juice. Hydrolysis with Viscozyme® L and Celluclast® 1.5 L by decreasing TDF could help release anthocyanins from the vacuoles of the pulp cells and thus increase a* values. Meanwhile, enzymatic hydrolysis with Pectinex Ultra Tropical® decreased the a* value,

although the decrease in TDF in this sample was most effective. Phenolic compounds in pomace are bound to dietary fiber; therefore, during the decomposition of dietary fiber, more phenolic compounds are released under enzymatic treatment. The decreased amount of phenolic content could also be due to the high temperature used to inactivate enzymes after hydrolysis [40]. Anthocyanins are sensitive to changes in temperature and pH [41]. The heating used during the inactivation of enzyme preparations may have harmed the stability of these compounds in the pomace. Azman et al. [42] also found a decrease in the anthocyanin content in BCP after heating up to 30 min at temperatures below 100 °C. This color change may also be due to the browning phenomenon that is a result of heat treatment [43]. Enzymatic hydrolysis using the Pectinex® Ultra Tropical enzyme complex may also degrade some of the anthocyanins in the pomace. Anthocyanins can be degraded in deglycosidation by β -glucosidase and oxidation by polyphenol oxidase or peroxidase [44]. Pectinex® Ultra Tropical may have exhibited secondary β -glucosidase activity, which could have contributed to the reduction of the red color.

Enzymatic treatment of BCP affected their technological properties in different ways (Table 2). In all cases, hydrolysis reduced the ORC (9.69–36.18%) and increased the WRC (12.45–36.95%) and WSI (16.4–48.9%) but had no statistically significant effect on bulk density. The most significant changes in technological properties were determined after the hydrolysis of pomace with Pectinex® Ultra Tropical and Viscozyme® L, while the least pronounced changes occurred after hydrolysis with Celluclast® 1.5 L. The observed differences in hydrolysis can be attributed to the specifics of enzyme action: Celluclast® 1.5 L hydrolyzes cellulose, while Pectinex® Ultra Tropical and Viscozyme® L, which are composed of enzyme complex such as pectinases and cellulases. It can be stated that hydrolysis with an enzyme complex had a greater influence on the changes in technological properties.

The determined ORC values of BCP were higher than those reported by Reißner et al. [29]—2 g/g d.w. It is worth noting that BCP in this study exhibited a higher ORC compared to other fruit, berry, or vegetable pomaces: chokeberry (1.74 g/g d.w.), blueberry (1.79 g/g d.w.), and elderberry (2.24 g/g d.w.) [45], apple—2.24 g/g d.w., carrot—2.44 g/g d.w., and beetroot—2.21 g/g d.w. [46]. Lingonberry pomace exhibited a comparable ORC (2.27–3.86 g/g), which was found to be influenced by the type of enzyme used for hydrolysis [32]. Variations in TDF content and the bulk density of the pomace (Tables 2 and 3) notably affected its ORC. Its overall charge density, which in turn influences the hydrophilic nature of its constituents and surface properties, is indicated by its bulk density. Generally, greater bulk density corresponds to reduced porosity and smaller particle size [27,47]. Various studies indicate that substances with high ORC may stabilize fat-emulsion-based products [48]. Choi et al. [49] investigated the influence of apple pomace on the quality of low-fat chicken sausages and found that reducing the fat content from 30% to 20% in sausages with added apple pomace led to lower cooking, fat, and moisture losses, as well as improved textural properties.

Gouw et al. [40] observed higher WRC in apple pomace (9.27 g/g d.w.), cranberry (8.70 g/g d.w.), blueberry (8.29 g/g d.w.), and raspberry (7.71 g/g d.w.). In another study, lingonberry pomace showed similar WRC (4.88–6.12 g/g), depending on the enzyme used in hydrolysis [32]. According to researchers, WRC primarily depends on particle size and surface characteristics, determining their electrostatic properties. Nemetz et al. [45] reported that WRC increases with increasing particle size. Gouw et al. [40], who examined the morphological characteristics of apple, cranberry, blueberry, and raspberry pomaces, found that apple pomace typically contains larger particles (fibers) and wider gaps between them, providing more sites for water binding. In contrast, raspberry pomace, which retains many seeds with higher cellulose and lignin content, had the fewest such sites and held less water.

Sezer et al. [31], studying the effect of enzymatic hydrolysis with cellulase from *T. reesei* on the technological properties of cherry pomace, found that after hydrolysis, WRC (from 10.12 to 9.13 g/g) and ORC (from 2.32 to 1.10 g/g) decreased. Meanwhile, solubility increased from 23.03% to 43.22%. According to Huang et al. [50], solubility is an indicator of the degradation of fibrous materials, resulting in the formation of low-molecular-weight compounds.

The effect of enzymatically treated BCP on emulsion stability is shown in Figure 3. Emulsion under static conditions was more stable than emulsions after heat treatment (30 min at 80 °C). The more stable emulsions (lowest creaming index) were prepared under static conditions with untreated BCP. The stability of emulsions strongly depended on the pH value, which increased with the increase in pH. The lowest stability was obtained using BCP hydrolysed with Celluclast 1.5 L. The emulsion's stability is affected by pomace components such as SDF, IDF, and protein content [51]. BCP modified with Celluclast® 1.5 L contained the lowest IDF content, which may significantly influence the creaming index. During hydrolysis, compounds with higher molecular weights are broken down into compounds with lower molecular weights, increasing the solubility of the compounds. Huang et al. [50] investigated the influence of carboxymethyl cellulose molecular mass on emulsions stabilized with whey protein isolate. Their study demonstrated that carboxymethyl celluloses with higher molecular weights were more effective in stabilizing emulsions compared to those with lower molecular weights. The stability of emulsions prepared with berry pomace may be attributed to two main mechanisms proposed in the literature. The first suggests that the finest particles within the insoluble fraction of the pomace adsorb at the oil–water interface, forming a protective layer around the oil droplets that inhibits coalescence. The second mechanism proposes that the insoluble fraction, in conjunction with the soluble components of the pomace—particularly endogenous proteins—increases the viscosity of the continuous phase, thereby enhancing the physical stability of the emulsion through reduced droplet mobility [51].

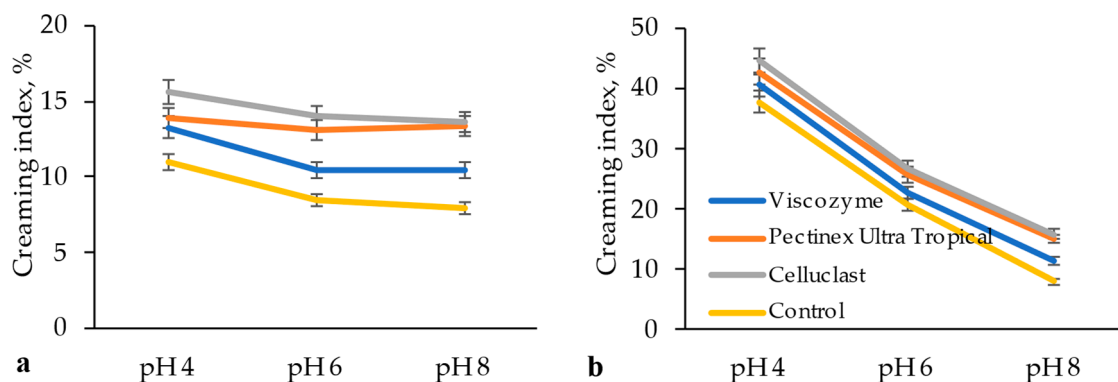


Figure 3. Stability of emulsions prepared with hydrolyzed BCP under static conditions (a) and after heat treatment (30 min at 80 °C) (b), depending on pH value. Mean \pm standard deviation values ($n = 3$).

Xu et al. [52] investigated the influence of pH on the emulsifying properties of polysaccharides, noting that the effect is dependent on the conformation of the polysaccharide. Anionic polysaccharides, such as pectin, are more susceptible to changes in pH. At acidic pH (e.g., pH 4), anionic polysaccharides form a dense film on the surface of oil droplets. A weaker and more easily ruptured film is formed at alkaline pH (e.g., pH 8). In contrast, neutral polysaccharides, such as cellulose, are less affected by pH. The reduced emulsion stability in acidic conditions indicates that the SDF fraction of BCP (mainly pectin) contributes less to emulsion stabilization compared to the IDF fraction (mainly cellulose).

4. Conclusions

The blackcurrant pomace (BCP) is a good source of nutritional components, especially dietary fiber (49.24 g/100 g d.w.), highlighting its potential use as a functional ingredient in food products. However, the potential of BCP to be valorized through enzymatic hydrolysis, particularly by modifying its dietary fiber composition, remains largely unexplored and underutilized. The enzymatic hydrolysis using different carbohydrases significantly affected not only the dietary fiber composition but also the technological properties of BCP. Hydrolysis using pectinolytic enzymes (Pectinex® Ultra Tropical and Viscozyme® L) mainly hydrolyzed SDF to mono- and disaccharides, while cellulolytic enzymes (Celluclast® 1.5 L) hydrolyzed IDF and increased the SDF content and improved the SDF/IDF ratio (1:3.5), which was reached close to the recommended one (1:2–3). Enzymatic hydrolysis decreased the ORC and increased the WRC of BCP. BCP treatment with different carbohydrases had a negative impact on the emulsion stabilizing properties. With increasing amounts of hydrolyzed IDF, the stability of emulsions decreased. The greatest changes in technological properties were observed after hydrolysis using Pectinex Ultra Tropical and Viscozyme L, which exhibit pectinolytic activity, and the least using Celluclast 1.5 L, which exhibits cellulolytic activity. Enzymatic hydrolysis using commercial carbohydrases highlights the potential for pomace valorization as a nutritional component with distinct technological properties depending on the enzyme used. However, some of the observed limitations—such as enzyme-specific effects on fiber solubility and functionality—may restrict the applicability of the ingredient in certain formulations. Future studies could explore alternative enzymatic strategies, including combinations of enzymes or extended hydrolysis times, to optimize pomace functionality for a broader range of food applications.

Results showed that using enzymatic treatment could improve BCP's dietary fiber composition and obtain functional ingredients with different technological properties, depending on the enzyme used. BCP, characterized by low ORC, could be a suitable additive for producing products intended for frying in oil, thereby indirectly reducing their caloric content and preventing a greasy mouthfeel. Due to its higher water retention capacity, hydrolyzed BCP could be recommended for further application in meat product formulation, as an additive to reduce heat treatment losses. This presents a promising direction for future research. Meanwhile, the recommended SDF/IDF ratio in BCP can be achieved using Celluclast 1.5 L for hydrolysis, which enhances the physiological potential of the pomace and broadens its applicability in the development of high-fiber functional food products. Further studies are needed to evaluate the effect of other carbohydrates on BCP or a combination of enzymatic hydrolysis and physical or chemical treatment. Understanding the structural changes that occur during hydrolysis can help tailor dietary fiber for specific health benefits and food applications. Exploring novel enzyme cocktails or sequential treatments may enhance the modification efficiency and broaden the functional potential of pomace fibers. Studies comparing various fruit pomaces under these conditions would provide valuable insights. Finally, investigating the functional impact of modified dietary fibers in specific food applications and assessing their physiological effects in vivo would help translate these findings into practical benefits.

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Abbreviations

The following abbreviations are used in this manuscript:

BCP	black currant pomace
TDF	total dietary fiber
SDS	soluble dietary fiber
IDF	insoluble dietary fiber
ORC	oil retention capacity
WSI	water solubility index
WRC	eater retention capacity

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