applied sciences

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Effect of Modification Methods on Composition and Technological Properties of Sea Buckthorn (*Hippophae rhamnoides* L.) Pomace

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Abstract

With the growth of the plant-based food sector, increasing amounts of by-products are generated. Sea buckthorn pomace (SBP), a by-product of juice and other manufacturing products, is rich in bioactive compounds such as phenolics, oligosaccharides, proteins, and dietary fiber. The aim of the study was to evaluate the impact of modification methods, such as enzymatic hydrolysis and supercritical carbon dioxide extraction (SFE-CO_2), on the chemical composition and technological properties of SBP. SBP and SBP obtained after SFE-CO_2 (SBP-CO_2) were enzymatically modified using Pectinex® Ultra Tropical, Viscozyme® L, and Celluclast® 1.5 L (Novozyme A/S, Bagsværd, Denmark). The SBP's main constituent was insoluble dietary fiber (IDF), followed by crude proteins and lipids (respectively, 58.7, 21.1 and 12.6 g/100 in d.m.). SFE-CO_2 reduced the lipid content (by 85.7%) in the pomace while increasing protein and TDF content. Enzymatic hydrolysis decreased the content of both soluble dietary fiber (SDF) and IDF, and increased the content of mono- and oligosaccharides as well as free phenolics, depending on the commercial enzyme preparation used in SBP and SBP-CO_2 samples. Celluclast® 1.5 L was the most effective in hydrolyzing IDF, while Viscozyme® L and Pectinex® Ultra Tropical were the most effective in degrading SDF. Enzymatic treatment improved water swelling capacity, water retention capacity, water solubility index, oil retention capacity of SBP and SBP-CO_2; however, it did not have a significant effect on the stability of the emulsions. Modification of SBP by SFE-CO_2 effectively increased WSC and WSI, however it reduced WRC. These findings highlight the potential of targeted modifications to enhance the nutritional and technological properties of SBP for functional food applications.

Keywords: sea buckthorn pomace; enzymatic hydrolysis; dietary fiber; oligosaccharides; technological properties

Academic Editor: Monika Gibis

Received: 30 June 2025
Revised: 24 July 2025
Accepted: 5 August 2025
Published: 7 August 2025

Citation: Kaminskytė, G.; Jagelavičiūtė, J.; Bašinskienė, L.; Syrpas, M.; Čižeikienė, D. Effect of Modification Methods on Composition and Technological Properties of Sea Buckthorn (*Hippophae rhamnoides* L.) Pomace. *Appl. Sci.* **2025**, *15*, 8722. https://doi.org/10.3390/app15158722

1. Introduction

During the processing of raw materials cultivated in agriculture, large quantities of by-products and waste are generated, which are still used inefficiently. Agricultural waste and by-products from the processing of plant raw material are widely utilized in animal feed production, while biomass residues are used for bioenergy production. However, due to the high content of valuable compounds in plant-based waste, their application in the production of higher-value food products or in the pharmaceutical and cosmetics industries would be both important and economically beneficial [1]. Biologically valuable

compounds can be extracted and recovered from plant-derived waste and processing by-products, transforming them into food ingredients, supplements, and/or functional food products [2–4].

Berry processing by-products are rich in biologically active components such as proteins and amino acids, dietary fibers and oligosaccharides, phenolic compounds, lipids and fatty acids, and vitamins [5]. With appropriate processing, these compounds can be used to develop products that not only possess improved functional properties but also provide greater health benefits [6].

Sea buckthorn (*Hippophae rhamnoides* L.) berries are rarely consumed fresh due to their sour and astringent taste; therefore, they are typically processed and used as ingredients in beverages, juices, jams, candies, and other products. These berries are rich in antioxidants, polyphenols, unsaturated fatty acids, tocopherols, and phytosterols [7–9]. To date, most of the research has focused on the analysis of polyphenolic extracts, with particular emphasis on the composition and bioactivity of their lipophilic and hydrophilic fractions [9–11]. However, the dietary fibers present in sea buckthorn pomace (SBP) may also serve as potential components in functional products for the food, pharmaceutical, and cosmetic industries providing low-cost raw materials for value-added products [12]. Comprehensive and systematic data on the composition of dietary fibers in SBP are lacking. For effective industrial application of these pomace residues, it is important to understand their technological properties. Literature data on the technological characteristics of SBP, particularly in relation to emulsion stabilization, are also scarce.

When assessing the health impact of dietary fibers, an important parameter is the ratio of SDF to IDF and the content of oligosaccharides as potential prebiotics. The recommended ratio of soluble to insoluble fiber is between 1:2 and 1:3, whereas most berry pomaces contain significantly higher amounts of insoluble fiber [13,14]. To increase the levels of soluble dietary fiber and oligosaccharides in berry pomace and to improve their technological functionality, various modification methods can be applied, including enzymatic hydrolysis [15,16], and others. No information has been found on the effects of supercritical CO_2 extraction (SFE-CO_2) on the fiber and technological properties of SBP, and data on the effects of enzymatic hydrolysis are limited. By applying modern technologies, it is possible to identify the most suitable method for modifying SBP to enhance its value and potential applications in food. The aim of this study was to evaluate the effects of modification methods–supercritical CO_2 extraction, and enzymatic hydrolysis—on the composition and technological properties of sea buckthorn (*Hippophae rhamnoides* L.) pomace.

2. Materials and Methods

2.1. Preparation and Enzymatic Treatment of Sea Buckthorn Pomace (SBP)

Fresh SBP was generously provided by the local company. The pomace was dried at 35–40 °C for 48 h to achieve a moisture content of about 4.17% and milled with a ZM 200 centrifugal mill (Retsch, Haan, Germany) to obtain particles smaller than 500 µm.

For the modification process, supercritical fluid extraction with carbon dioxide (SFE-CO_2) was employed using a Helix extraction system (Applied Separation, Allentown, PA, USA) under the following optimized conditions: 45 MPa pressure, 50 °C temperature, 240 min extraction time, and a CO_2 flow rate of 2 SL/min. The resulting material, referred to as SBP-CO_2, was collected and used for subsequent analyses. These extraction parameters were selected based on prior optimization studies conducted by Kitrytė et al. [17].

Enzymatic modification of the SBP was performed using commercial enzymes from Novozymes A/S (Bagsvaerd, Denmark): Viscozyme® L (100 FBG/g), Pectinex® Ultra Tropical (5000 PECTU/g), and Celluclast® 1.5 L (700 EGU/g). Each enzymatic treatment was carried out by combining 2.5 g of sample with 37.5 mL of distilled water and 0.25 mL

of the respective enzyme. The enzymatic hydrolysis was conducted at 50 °C for 1 h with continuous shaking at 200 rpm. Enzymatic activity was terminated by heating the mixture to 90 °C for 20 min, followed by cooling to room temperature (~20 °C).

For saccharide profiling (mono-, di-, and oligosaccharides), the hydrolyzed mixture was centrifuged at 8000 rpm for 20 min. The supernatant was collected, frozen at –80 °C, and freeze-dried using a freeze dryer (North Salt Lake, UT, USA). To assess SDF and IDF and evaluate technological properties, the whole hydrolyzed suspension was frozen at –80 °C and subsequently freeze-dried (no additional separation was applied). Freeze-dried SBP was stored in airtight containers at 4 °C until further use.

2.2. Proximate Composition and Dietary Fiber Determination

The moisture mass was quantified by drying 1 g of the SBP at 105 °C till a stable mass was obtained, according to the guidelines of the AOAC Method 925.10 [18]. Lipid content was assessed through Soxhlet extraction, utilizing 3 g of SBP and hexane as the solvent over a 3 h period, according to AOAC Method 948.22 [18]. Protein content was determined using the Kjeldahl equipment on 1 g of SBP, applying a nitrogen-to-protein conversion factor of 6.25, according to AOAC Method 978.04 [18]. To determine ash content, 2 g of SBP was first charred for 30 min, then incinerated in a muffle furnace at 625 °C for 2 h, following AOAC Method 930.05 [18]. Total dietary fibers (TDF), SDF, and IDF were measured using a commercial enzymatic-gravimetric assay kit purchased from Megazyme International (Wicklow, Ireland) according to the manufacturer's instructions [19].

2.3. Saccharide Profiling Using High-Performance Liquid Chromatography

The saccharide content was analyzed by dissolving 10 mg of the freeze-dried water-soluble SBP fraction in 1 mL of ultrapure water (Millipore, Darmstadt, Germany), resulting in a final concentration of 10 mg/mL. The identification and quantification of mono- and disaccharides were performed according to the procedure described by Bytautaite et al. [20], using a Thermo Scientific Ultimate 3000 HPLC system equipped with a RefractoMax 521 refractive index detector (Thermo Fisher Scientific, Waltham, MA, USA).

Saccharides were separated using two serially connected columns, SUGAR KS-802 and KS-801 (both with an internal diameter of 8.0 mm and a length of 300 mm), with ultrapure water serving as the mobile phase. The separation was performed under isocratic conditions at a constant flow rate of 0.5 mL/min, with the column temperature maintained at 80 °C. Each sample (10 µL) was injected and analyzed over a 60 min runtime. Data acquisition and chromatographic analysis were conducted using Chromeleon 7 software (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) in modified pomace was determined using a the Folin–Ciocalteu colorimetric assay described by Singleton et al. [21] with slight modifications. Extraction was performed by suspending 1 g of pomace in 10 mL of an acidified methanol solution (0.1% HCl in methanol, v/v), followed by incubation in the dark at 4 °C overnight. After that 1 mL of the resulting extract was combined with 5 mL of diluted Folin–Ciocalteu reagent (1:9, v/v) and 4 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark for 30 min, after which absorbance was recorded at 765 nm using a spectrophotometer (Thermo Electron LED GmbH, Langenselbold, Germany). The TPC was quantified based on a gallic acid calibration curve (R^2 = 0.999) and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry pomace.

2.5. Hydration Properties

Water swelling capacity (WSC) and water retention capacity (WRC) was assessed following a modified version of the method described by Yu et al. [22]. 0.200 ± 0.001 g of sample was placed into a centrifuge tube, and its initial volume was recorded. The sample was then combined with 6 mL of distilled water and kept at 21 °C for 18 h. After this period, the final hydrated volume was measured. WSC was determined using the following equation:

$$\text{WSC (mL/g)} = (V_1 - V_0)/M \quad (1)$$

where V_0 is the initial volume of the dry sample (mL), V_1 is the volume after hydration (mL), and M represents the sample mass expressed as dry matter (g).

After WSC evaluation sample was centrifuged at 5000 rpm ± 0.001 (Microcen 23, Ortoalresa, Madrid, Spain), and the remaining material was weighed, dried at 105 ± 2 °C till a stable mass was obtained, and weighed again. The WRC was estimated using equation:

$$\text{WRC (g/g)} = (M_1 - M_2)/M_2 \quad (2)$$

where M_1 is the mass of wet residue (g), and M_2 is the mass after drying (g).

The supernatant collected after centrifugation was used to determine the water solubility index (WSI). It was dried at 105 ± 2 °C till a stable mass was obtained. WSI was estimated as:

$$\text{WSI (\%)} = (M/M_0) \times 100 \quad (3)$$

where M is the mass of the soluble solids after drying (g), and M_0 is the dry matter content of the original sample (g).

2.6. Oil Retention Capacity (ORC)

The ORC was assessed following to a modified procedure based on Yu et al. [22]. Sample (0.200 ± 0.001 g) was carefully vortexed with 2 g of sunflower oil and kept at 21 °C for 1 h following the centrifugation at 3000 rpm ± 0.001 for 10 min. The oil was poured, and the residue was weighed. The ORC was estimated by equation:

$$\text{ORC (g/g)} = (M_1 - M_0)/M_0 \quad (4)$$

where M_1 is the mass of the residue (g), and M_0 is the dry matter content of the original sample (g).

2.7. Bulk Density (BD)

The BD was measured following the method of Jagelavičiūtė et al. [23]. A known mass of sample (0.200 ± 0.001 g) was added to a graduated tube, which was tapped 20 times on a flat surface to compact the sample, and the final volume was noted. BD was calculated using equation:

$$\text{BD (g/mL)} = M/V \quad (5)$$

where M is the sample's dry mass (g), and V is the packed volume (mL).

2.8. Color Analysis

Color parameters were assessed using a Konica Minolta colorimeter (Tokyo, Japan) operating within the CIE Lab* color system. Prior to each measurement session, the instrument was calibrated using standard white and black calibration plates provided by the manufacturer to ensure measurement accuracy. In this model, the *L** value represents lightness, ranging from 0 (black) to 100 (white), the *a** coordinate reflects the green to red spectrum, and the *b** coordinate indicates the blue to yellow range. The BCP sample was

evenly spread across a plate to ensure uniform coverage. Subsequently, color measurements were taken at three randomly selected points from the bottom area of the plate. To assess color differences, the ΔE value was determined following the methodology described by Sivam et al. [24]. Chroma and ΔE were calculated using Equations (6) and (7):

$$\text{Chroma} = \sqrt{a2 + b2} \quad (6)$$

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

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

2.9. Emulsion Preparation and Stability Assessment

To prepare emulsions, 0.160 ± 0.001 g of SBP was homogenized with 8 mL of distilled H_2O and 8 g of sunflower oil in a graduated tube. Emulsification was carried out at 10,000 rpm for 5 min with a high-speed homogenizer (IKA® T-25 digital, Ultra-Turrax, Staufen, Germany). Emulsion stability was evaluated by assessing creaming index under different conditions following 30 min of storage at 22 °C (static conditions), or after treatment at 80 °C for 30 min (thermal stability). Creaming index was estimated as described by Keršienė et al. [25] following Equation (8):

$$\text{Creaming index (\%)} = (\text{SV}/\text{TV}) \times 100 \quad (8)$$

where SV is the volume of the serum phase (mL), and TV is the total emulsion volume (mL).

Thermal stability was assessed by placing the emulsions in a water bath at 80 °C for 30 min, followed by creaming index determination. Furthermore, emulsion stability was monitored after–12 days of storage at 4 °C, and creaming index was calculated and expressed following Equation (8). To assess pH effects on emulsion stability, buffer solutions (0.1 M citric acid and 0.2 M Na_2HPO_4) were used instead of water to prepare emulsions with target pH values of 4.0, 6.0, and 8.0.

2.10. Statistical Analysis

Each measurement was carried out in triplicate. The mean values and standard deviations were determined using Microsoft Excel 2019 (Microsoft Corporation, Albuquerque, NM, USA). For statistical comparisons, one-way analysis of variance (ANOVA) was applied using Statgraphics Centurion 19 software. Tukey's honest significant difference (HSD) test was used for post hoc analysis, and differences were considered statistically significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Chemical Composition of SBP and SBP-CO_2

Fractionated extraction approaches, including SFE-CO_2 and complementary techniques, are increasingly employed in biorefinery processes to selectively isolate lipophilic and polar bioactive fractions from fruit and berry pomace, whereas the residual biomass—despite being rich in fiber and protein—often receives less attention in terms of valorization [26,27]. In this study the proximate composition of SBP and SBP residues after SFE-CO_2 is shown in Table 1. The main constituents of SBP are TDF followed by protein and fat content. Dienaitė et al. reported a similar chemical composition of the SBP except for the lipid content (20.78% d.m.), which was 66% higher than in the analyzed SBP [10]. The lipid content in the SBP can vary from 1.8 to 29.1%, depending on the amount of seed, pulp and peel fractions in the raw material. The observed differences in the chemical composition of the pomace may depend on climatic growing conditions, berry size, degree of maturity,

variety characteristics, storage conditions and processing methods [28]. Application of SFE-CO_2 resulted in an 85.7% reduction in the lipid content of the SBP-CO_2 after extraction. In this regard, the protein content increased by 20.1% and the TDF content increased by 10%. In another study, SFE-CO_2 extraction of black currant pomace not only resulted in a nearly complete defatting of the material, leaving less than 0.8% residual lipids, but also led to a significant 30% increase in protein content [29]. The efficiency of SFE-CO_2 depends on several extraction parameters (pressure, time, temperature, sample particle size, total solvent type and amount). Therefore, the process should be optimized for each individual plant material before its application for industrial use [30].

Table 1. Proximate composition of SBP and SBP-CO_2, g/100 g d.m.

Parameter	SBP	SBP-CO_2
Moisture, %	4.17 ± 0.04 [a]	5.25 ± 0.05 [b]
Crude protein	21.09 ± 0.28 [a]	25.45 ± 0.41 [b]
Lipids	12.57 ± 0.39 [a]	1.80 ± 0.20 [b]
Ash	1.38 ± 0.01 [a]	1.53 ± 0.03 [b]
TDF	63.61 ± 0.79 [a]	70.65 ± 0.56 [b]
IDF	58.69 ± 0.69 [a]	63.93 ± 0.68 [b]
SDF	4.92 ± 0.1 [a]	6.72 ± 0.44 [b]
SDF/IDF ratio	1:12	1:9.5
[1] Mono- and oligosaccharides	1.35 ± 1.22 [a]	0.57 ± 0.45 [a]

[1] Mono- and oligosaccharides = 100 − (crude protein + lipids + ash + TDF). Mean ± standard deviation values in row with different lowercase letters are significantly different ($p < 0.05$).

The SDF/IDF ratio is important for the nutritional value and technological properties of the pomace, such as water retention capacity and viscosity. From a nutritional point of view, the SDF/IDF ratio should be close to 1:2–1:3 [15,16]. Considering that the SDF/IDF ratio obtained during the study (1:12 and 1:9.5) does not correspond to the recommended one, additional processing of the SBP is required to increase the SDF content.

3.2. Enzymatic Hydrolysis Effect on Dietary Fiber Composition of SBP and SBP-CO_2

Enzymatic hydrolysis of berry and fruit pomace is often used to increase its SDF and oligosaccharide content. Therefore, the present study investigated the effect of enzymatic treatment on the changes in the fiber content of SBP and SBP-CO_2. Since pectin, hemicelluloses and cellulose predominate in the polysaccharides of berry fiber, the main enzymatic activities of commercial enzymes selected for hydrolysis were cellulase (Celluclast® 1.5 L), endo-β-glucanase (Viscozyme® L), pectin lyase and pectinase (Pectinex® Ultra Tropical). The dietary fiber content and composition changes after hydrolysis are shown in Table 2.

Table 2. Enzymatic hydrolysis effect on the amount and composition of dietary fibers and total phenolic content (TPC) in SBP and SBP-CO_2.

	SBP				SBP-CO_2			
	Control	Viscozyme® L	Pectinex® Ultra Tropical	Celluclast® 1.5 L	Control	Viscozyme® L	Pectinex® Ultra Tropical	Celluclast® 1.5 L
TDF, g/100 g d.m.	63.61 ± 0.79 [e]	55.85 ± 0.31 [b]	54.86 ± 0.31 [b]	52.9 ± 0.31 [a]	70.65 ± 0.56 [e]	63.09 ± 0.28 [e]	61.37 ± 0.21 [d]	59.19 ± 0.67 [c]
SDF, g/100 g d.m.	4.92 ± 0.1 [c]	2.90 ± 0.02 [a]	3.52 ± 0.31 [ab]	4.68 ± 0.62 [bc]	6.72 ± 0.44 [d]	4.22 ± 0.48 [c]	4.96 ± 0.12 [c]	6.41 ± 0.53 [d]
IDF, g/100 g d.m.	58.69 ± 0.69 [d]	52.95 ± 0.61 [c]	51.34 ± 0.59 [b]	48.22 ± 0.31 [a]	63.93 ± 0.68 [f]	58.87 ± 0.08 [d]	56.41 ± 0.31 [e]	52.78 ± 0.02 [c]
SDF/IDF ratio	1:12	1:18.2	1:14.6	1:10.3	1:9.5	1:13.9	1:11.4	1:8.2
TPC, mg GAE/g	5.73 ± 0.02 [e]	5.96 ± 0.02 [f]	6.05 ± 0.01 [g]	6.15 ± 0.02 [h]	4.64 ± 0.01 [a]	4.81 ± 0.01 [b]	4.85 ± 0.01 [c]	4.92 ± 0.01 [d]

Different lowercase letters (a–h) within a row indicate statistically significant differences between values ($p < 0.05$).

The hydrolysis with all commercial enzymes resulted in a decrease in TDF content. The enzymatic treatment with Viscozyme® L had the lowest effect on the IDF content of

SBP and SBP-CO_2 (IDF decreased by 12.2% and 10.7%, respectively). While treatment with Celluclast® 1.5 L hydrolyzed the most IDF (16.8% and 16.2% for SBP and SBP-CO_2, respectively). In SBP and SBP-CO_2 samples, the most pronounced degradation of SDF was achieved with Viscozyme® L, resulting in reductions of 41.05% and 37.2%, respectively. In contrast, hydrolysis with Celluclast® 1.5 L had the least effect, with corresponding reductions of only 4.9% and 4.6%. The results demonstrated that enzyme preparation mainly composed of cellulose-degrading enzyme—cellulase (Celluclast® 1.5 L) was the most effective in hydrolyzing IDF, while enzyme preparations containing pectinases (Viscozyme® L and Pectinex® Ultra Tropical) were the most effective in degrading SDF. The polysaccharide hydrolysis using Celluclast® 1.5 L had a positive effect on the SDF/IDF ratio, increasing it from 1:12 to 1:10.3 and 1:8.2 in SBP and SBP-CO_2 samples, respectively. The increase in SDF/IDF was reported in other pomace after enzymatic treatment with cellulase [15,16,31]. The partial removal of lipophilic compounds through SFE-CO_2 extraction had no notable impact on the efficiency of enzymatic hydrolysis.

The observed changes in dietary fiber fractions can be directly related to the site of enzymatic action and the substrate specificity of the enzymes used. The synergistic activity of enzyme complexes containing pectinases, cellulases, and β-glucosidases facilitates the disruption of pectin and lignin networks, allowing better enzymatic access to cellulose-rich regions [32,33]. In this study, Viscozyme® L—a multi-enzyme preparation containing polygalacturonases, cellulase, hemicellulases, xylanases, β-glucanase, and arabinose. Pectinex® Ultra Tropical, composed of pectin lyases, polygalacturonases, β-glucanase, and cellulase, showed complementary activity. Comparable reductions in dietary fiber content after Viscozyme® L treatment have been reported by Mrabet et al. [34], while Yoon et al. [35] observed increased alcohol-soluble fiber following enzymatic treatment of carrot pomace. In other studies, enzymatic hydrolysis with cellulase increased SDF content [15,16,31]; however, the effect depends on factors such as raw material properties, moisture content, and enzyme dosage. Nguyen et al. [36] observed a decrease in SDF in wheat bran after cellulase treatment, which was associated with the enhanced depolymerization of dietary fiber and increased release of low-molecular-weight carbohydrates at higher enzyme levels.

The enzymatic hydrolysis of fiber components such as hemicellulose, cellulose and pectin produce mono- and oligosaccharides, which have an important physiological and technological properties [37]. To further elucidate the results of enzymatic hydrolysis, an analysis of the mono- and oligosaccharide profiles of SBP was conducted (Figure 1). Enzymatically hydrolyzed SBP was selected for this analysis due to the slightly higher decrease in TDF and IDF content.

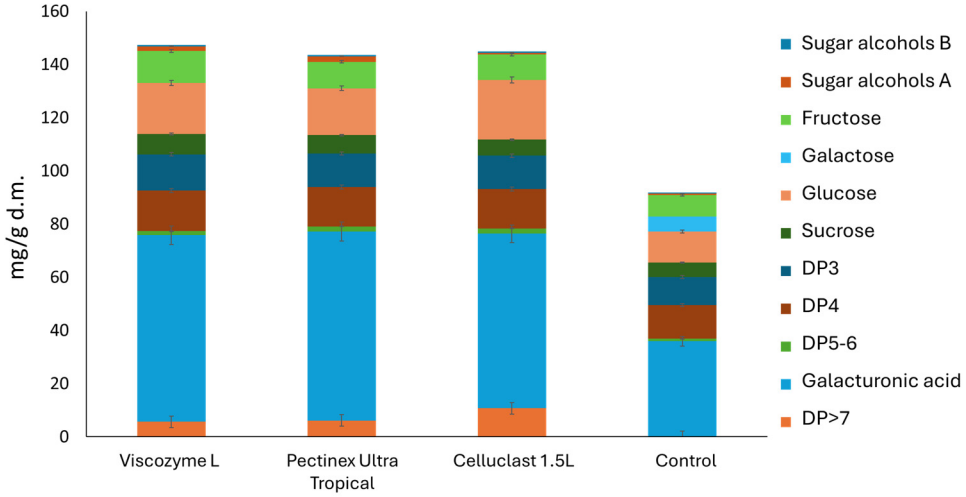

Figure 1. Effect of enzymatic hydrolysis on the saccharide profile of SBP.

Carbohydrate analysis by HPLC showed that the main saccharides in SBP were galacturonic acid (35.9 mg/g d.m.), DP4 (12.6 mg/g d.m.), glucose (11.6 mg/g d.m.), DP3 (10.6 mg/g d.m.), as well as slightly lower amounts of fructose, galactose, sucrose, oligosaccharides consisting of 5–6 monosaccharide units (8 mg/g d.m.) and sugar alcohols A and B were found. No oligosaccharides consisting of more than 7 monosaccharide units were detected in the control sample, while after hydrolysis with Viscozyme® L, Pectinex® Ultra Tropical and Celluclast® 1.5 L they were obtained of 5.6 mg/g d.m., 6.1 mg/g d.m., 10.7 mg/g d.m., respectively. Oligosaccharides consisting of 5–6 monosaccharide units were the most abundant oligosaccharides found in the samples hydrolyzed with Pectinex® Ultra tropical and Celluclast® 1.5 L (47.36% increase compared to control). DP4 and DP3 were the most abundant in the sample hydrolyzed with Viscozyme® L (17.64% and 22.05% increase compared to control).

Sojka et al. [38] investigated the levels of sucrose, glucose and fructose in the blackcurrant pomace, which were lower than those obtained from SBP. Kitryte et al. [30] investigated the effect of chokeberry pomace hydrolysis with Viscozyme® L on changes in glucose and fructose content, reporting an increase of 4–63% in glucose and 35–141% in fructose levels. Hydrolysis of the berry pomace with cellulolytic and xylanolytic enzymes increased the release of carbohydrates from the cell wall. These results may be explained by the partial disruption of the cell wall through the enzymatic hydrolysis process, which contributes to the release of carbohydrates from the cell matrix. Various factors such as growing conditions, crop year, cultivar, ripening, changes in the juicing process and the testing methods used may influence the carbohydrate composition of berries, their juices and pomace. Wilkowska et al. [39] demonstrated that enzymatic treatment of apple pomace with commercial cellulases and pectinases increased the yield of oligosaccharides. Preparations rich in higher-order oligosaccharides (DP $\geq$ 7) exhibited stronger bifidogenic effects and influenced short-chain fatty acid profiles, while even lower-degree oligomers (DP 2–6) contributed to the overall prebiotic potential observed across the DP 2–10 range. Jaouhari et al. [40] reported that the breakdown of IDF promoted the generation of oligosaccharides. The authors also observed that the enzymatic cocktail acted synergistically through cellulase and hemicellulase activities, resulting in maximal increases in glucooligosaccharides and fructooligosaccharides concentrations. In another study, enzymatic hydrolysis increased oligosaccharide content in orange and apple bagasse, particularly when combined with physical pre-treatment. Although techno-functional properties and phenolic content decreased, the concentration of bioactive compounds increased, yielding materials with elevated oligosaccharide levels and improved techno-functional and health-related properties [41].

The TPC in modified SBP extracts varied from 4.64 to 6.15 mg GAE/g. Literature reports a wide variation in TPC values across different sea buckthorn varieties. Wu et al. [42] reported that SBP extracted with 75% ethanol yielded the highest TPC, reaching 42.86 $\pm$ 0.73 mg GAE/g, whereas Luntraru et al. [11] observed a TPC value of 32.55 $\pm$ 0.86 mg GAE/g d.m. Similarly, depending on the drying conditions, the TPC of SBP has been shown to vary from 19.88 $\pm$ 0.20 to 28.56 $\pm$ 0.24 mg GAE/g d.m., further emphasizing the influence of processing methods on phenolic compound [43].

Free TPC increased in all extracts after enzymatic hydrolysis. The differences in phenolic compounds between samples hydrolyzed with different enzymes were statistically significant ($p < 0.05$). The highest levels of phenolic compounds were found in the extracts of SBP and SBP-CO_2 hydrolyzed with Celluclast® 1.5 L (with an increase by 7.32% and 6.03%, respectively). Some of the phenolic compounds are components of the plant cell wall and are released during the hydrolysis process when the fibers are degraded, making them easier to detect [44]. This explains the increase in TPC in the samples after hydrolysis.

3.3. Technological Properties of Enzymatically Modified SBP and SBP-CO_2

The technological properties (oil retention capacity (ORC), the swelling (WSC) and water retention capacity (WRC), the water solubility index (WSI), and the bulk density) of enzymatically modified SBP and SBP-CO_2 are shown in Table 3.

Table 3. Technological properties of enzymatically modified SBP and SBP-CO_2.

	SBP				SBP-CO_2			
	Control	Viscozyme® L	Pectinex® Ultra Tropical	Celluclast® 1.5 L	Control	Viscozyme® L	Pectinex® Ultra Tropical	Celluclast® 1.5 L
ORC, g/g d.m.	1.63 ± 0.06^{a}	2.80 ± 0.16^{e}	2.16 ± 0.05^{d}	3.08 ± 0.05^{f}	1.72 ± 0.03^{ab}	1.80 ± 0.26^{abc}	1.79 ± 0.2^{b}	2.01 ± 0.2^{c}
WSC, mL/g d.m.	2.61 ± 0.46^{a}	3.0 ± 0.02^{a}	3.30 ± 0.58^{ab}	3.83 ± 0.29^{b}	3.81 ± 0.31^{b}	4.47 ± 0.01^{c}	4.50 ± 0.01^{c}	4.69 ± 0.30^{c}
WRC, g/g d.m.	4.67 ± 0.21^{b}	5.24 ± 0.15^{cd}	5.12 ± 0.21^{cd}	5.59 ± 0.26^{d}	4.20 ± 0.05^{a}	4.63 ± 0.02^{b}	4.59 ± 0.17^{b}	4.94 ± 0.12^{c}
WSI, %	12.1 ± 0.3^{a}	15.4 ± 0.5^{b}	15.2 ± 0.4^{b}	12.6 ± 0.3^{b}	18.7 ± 0.1^{c}	20.2 ± 0.6^{d}	19.9 ± 0.3^{d}	18.9 ± 0.1^{c}
Bulk density, g/mL	0.34 ± 0.2^{ab}	0.31 ± 0.1^{a}	0.29 ± 0.1^{a}	0.32 ± 0.1^{a}	0.48 ± 0.1^{c}	0.38 ± 0.1^{b}	0.35 ± 0.1^{a}	0.33 ± 0.1^{a}
L^*	57.77 ± 0.29^{d}	49.90 ± 0.01^{a}	52.52 ± 0.01^{b}	56.45 ± 0.04^{c}	59.38 ± 0.01^{e}	62.01 ± 0.03^{h}	60.91 ± 0.08^{f}	61.60 ± 0.09^{g}
a^*	4.34 ± 0.04^{a}	5.87 ± 0.00^{e}	5.24 ± 0.01^{d}	4.66 ± 0.01^{b}	4.61 ± 0.02^{c}	4.72 ± 0.01^{d}	4.29 ± 0.07^{a}	4.37 ± 0.01^{b}
b^*	22.72 ± 0.12^{d}	21.06 ± 0.02^{a}	26.55 ± 0.01^{e}	22.17 ± 0.02^{c}	26.08 ± 0.03^{a}	27.33 ± 0.02^{c}	26.80 ± 0.24^{b}	26.87 ± 0.05^{b}
ΔE	-	8.19 ± 0.02^{d}	6.56 ± 0.00^{c}	1.47 ± 0.05^{a}	-	2.91 ± 0.04^{c}	1.73 ± 0.18^{a}	2.37 ± 0.13^{b}
Chroma	23.13 ± 0.14^{c}	21.87 ± 0.03^{a}	27.06 ± 0.01^{d}	22.65 ± 0.02^{b}	26.48 ± 0.04^{a}	27.74 ± 0.03^{c}	27.14 ± 0.30^{b}	27.23 ± 0.07^{b}

Different lowercase letters (a–h) within a row indicate statistically significant differences between values ($p < 0.05$).

The ORC of all hydrolyzed samples increased compared to the enzymatically untreated SBP and SBP-CO_2. Enzymatic hydrolysis with Celluclast® 1.5 L had the most pronounced effect on ORC. Enzymatic hydrolysis with this enzyme also led to the highest increase in WSC for SBP and SBP-CO_2 (45.98% and 23.10%, respectively) compared to the untreated samples. While enzymatic treatment with other enzymes also increased WSC values relative to the control sample: Viscozyme® L by 14.94% (SBP) and 17.32% (SBP-CO_2), and Pectinex® Ultra Tropical by 26.43% (SBP) and 18.11% (SBP-CO_2). The highest increase in WRC was observed in SBP treated with Celluclast® 1.5 L (19.70%), whereas the lowest increase was found in samples hydrolyzed with Pectinex® Ultra Tropical (9.63%).

The changes in dietary fiber content and composition in SBP and SBP-CO_2 have impacts for the technological properties of pomace. The ORC, WRC and WSC increased with decreased IDF content. Li et al. [45] reported that the observed increases in WSC and WRC following hydrolysis can be attributed to the enzymatic cleavage of polysaccharide chains, particularly when cellulase-containing enzymes are used. These enzymes disrupt β (1→4) glycosidic linkages between glucose units, leading to the depolymerization of polysaccharides. This process destroys the integrity of the cell wall, reduces particle size, and increases the surface area of the material. In this study, enzymatic treatment resulted in a decrease in bulk density, suggesting an increase in matrix porosity and a looser structure. Such structural modifications are consistent with fiber depolymerization and the formation of lower molecular weight fragments, which can affect water and oil retention properties under centrifugal force [46,47]. Consequently, due to the enhanced surface tension, greater amounts of water can be retained within the fiber's capillary structures in SBP [45]. As reported by Nemetz et al. [48], an increase in particle size tends to enhance WRC, likely due to the improved capacity for capillary water entrapment. Gouw et al. [49] demonstrated that apple pomace, composed of larger fibrous particles and greater pore volume, displayed superior water-holding performance compared to raspberry pomace, which contains a higher proportion of seeds and lignified tissues, limiting its hydrophilic interactions.

Also, Schmid et al. [50] found that increased solubility is often associated with elevated levels of water-soluble pectin and hemicellulose, which are enhanced through enzymatic hydrolysis of pomace.

The applied modifications significantly influenced the color characteristics of SBP (Table 3). Among the enzymatically hydrolyzed SBP samples, those treated with Viscozyme® L and Pectinex® Ultra Tropical exhibited the greatest increase in ΔE (total color difference).

Pomace hydrolyzed with Viscozyme® L showed the lightest color (highest L^* value), the most pronounced green–red color (a^*), but the least pronounced blue–yellow color (b^*) and the lowest chroma (color intensity). In contrast, SBP hydrolyzed with Pectinex® Ultra Tropical demonstrated the highest b^* value and greatest chroma. This effect can be attributed to the breakdown of polysaccharides bound to color and pigment compounds during hydrolysis, which often results in a reduction in color intensity. Similarly, among enzymatically hydrolyzed SBP-CO_2 samples, those treated with Viscozyme® L exhibited the most significant color changes (highest ΔE), characterized by the lightest color (L^*), the most intense a^* and b^* values, and the highest chroma.

The effect of enzymatically treated SBP and SBP-CO_2 on emulsion stability under static and thermal conditions are shown in Figure 2. When comparing the phase separation of emulsions stabilized with untreated (control) and enzymatically hydrolyzed SBP and SBP-CO_2, it was observed that the emulsion stability slightly decreased; however, the changes were not statistically significant.

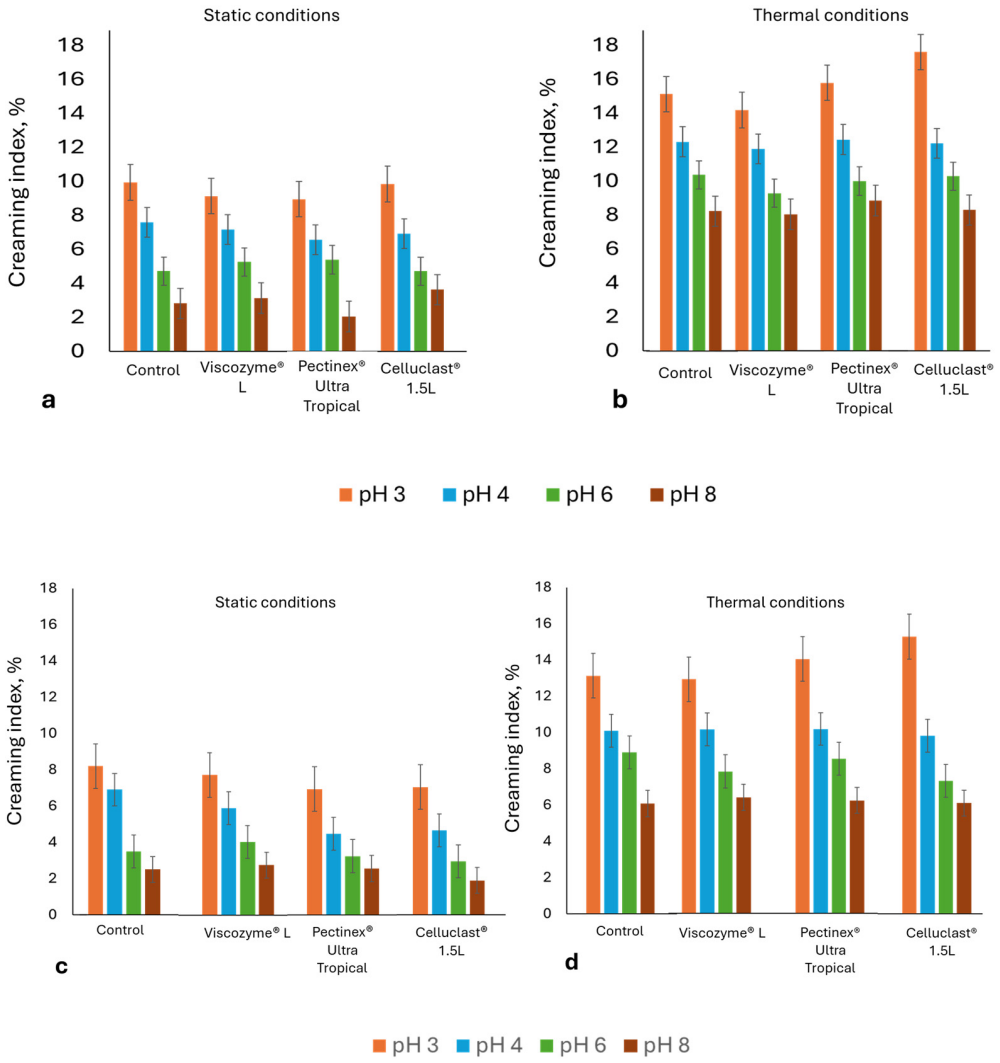

Figure 2. Stability of emulsions prepared with hydrolyzed SBP (**a**,**b**) and SBP-CO_2 (**c**,**d**) under static conditions (**a**,**c**) and after heat treatment at 80 °C for 30 min (**b**,**d**), depending on pH value. Mean ± standard deviation values (n = 3).

Since thermal processing (e.g., heating to 80 °C) is commonly applied in the production of various food products, it is crucial to evaluate the thermal stability of emulsions. Heating can negatively impact emulsion stability, particularly when effective stabilizing agents are

absent or insufficient [51,52]. Thermal treatment further reduced emulsion stability, as indicated by an increased creaming index for all samples. The lowest stability was observed at pH 3 in emulsions stabilized with SBP and SBP-CO_2 hydrolyzed by Celluclast® 1.5 L; however, there was no statistically significant difference compared to pH 4 and 6. Emulsion stability was improved with increasing pH, reaching the highest stability (lowest creaming index) at pH 8.

Evaluation of emulsion stability during 12 days of storage (Figure 3) revealed that most emulsions with enzymatically hydrolyzed SBP-CO_2 exhibited higher stability compared to those prepared with enzymatically hydrolyzed SBP. When assessing the influence of pH on the stability of emulsions with enzymatically treated SBP, the highest stability was observed at a pH of 8, while the lowest stability was observed at a pH of 3.

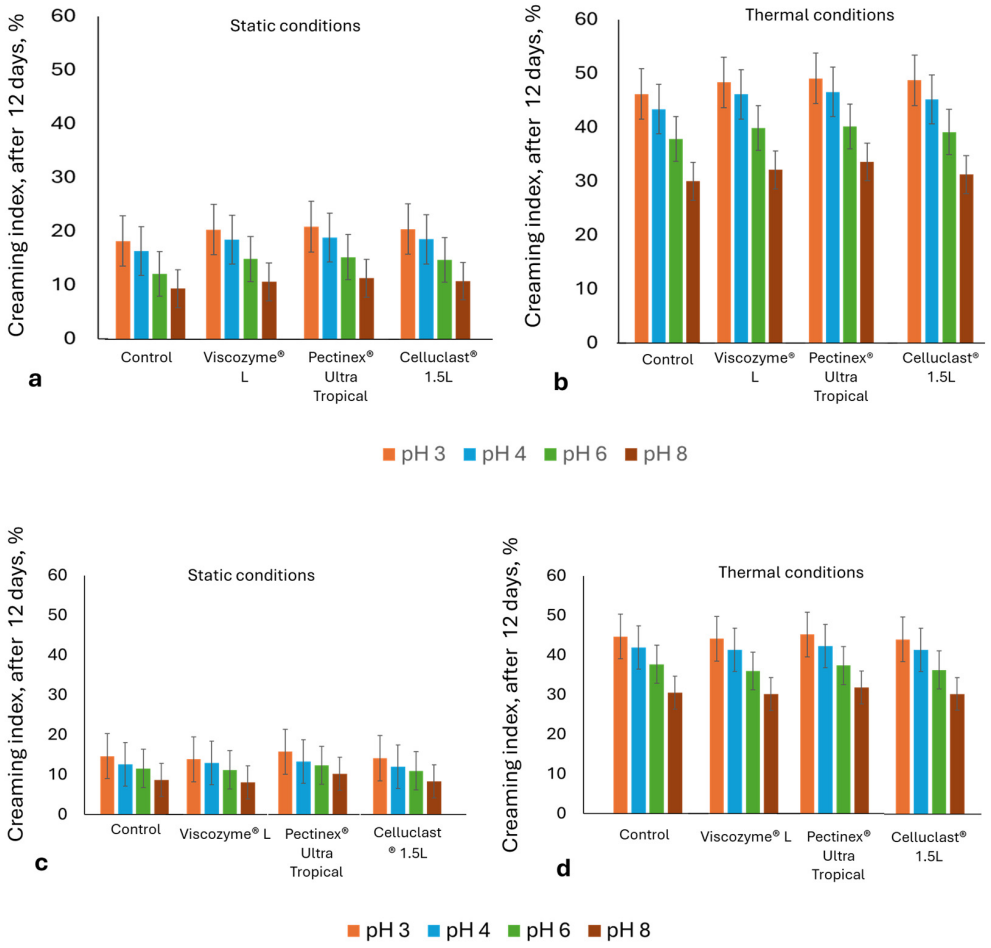

Figure 3. Stability of emulsions prepared with hydrolyzed SBP (**a**,**b**) and SBP-CO_2 (**c**,**d**) under static conditions (**a**,**c**) and after heat treatment at 80 °C for 30 min (**b**,**d**), depending on pH value after 12 days of storage at 4 °C. Mean ± standard deviation values (n = 3).

Huc-Mathis et al. [53] found in their study of Pickering emulsions based on food by-products that the bulk density of by-products, the WRC and the IDF content had the most significant influence on the stability of the emulsions. In the study, apple pomace had the highest bulk density, the lowest IDF content and the lowest WRC when compared to by-products from oat bran processing, and had the best emulsion stabilization properties. Other studies have also reported a reduced emulsion stability with modified berry pomace compared to untreated pomace [16,31]. The sample modified with Celluclast® 1.5 L exhibited the lowest IDF content, which may have contributed to a higher creaming index. Hydrolysis leads to the degradation of high-molecular-weight compounds (IDF) into

smaller molecular entities (oligosaccharides), thereby enhancing their solubility. In a study by Huang et al. [54], the effect of carboxymethyl cellulose molecular weight on the stability of emulsions stabilized with whey protein isolate was explored. The findings revealed that carboxymethyl celluloses with higher molecular weights provided superior emulsion stabilization compared to those with lower molecular weights. The stabilizing properties of berry pomace in emulsions are generally explained by two main mechanisms. First, the interaction between insoluble and soluble fractions—such as pectins, saccharides, and proteins—can increase the viscosity of the aqueous phase, leading to improved emulsion stability. Second, fine particles from the insoluble fraction may adsorb at the oil–water interface, forming a particulate layer that inhibits droplet coalescence and, thus, contributes to physical stabilization of the emulsion [55,56].

In this study, the pH value had a greater influence on the emulsion-stabilizing capacity of pomace than enzymatic hydrolysis. An increase in pH was associated with improved emulsion stability, suggesting that pH-induced changes in molecular charge and solubility played a more prominent role than enzymatic modifications. The emulsifying properties of polysaccharides are influenced by pH, as demonstrated by Xu et al. [57], who highlighted the role of polysaccharide conformation in this process. Anionic polysaccharides like pectin are particularly responsive to pH shifts. At low pH values (e.g., pH 4), they tend to form compact, cohesive films around oil droplets, whereas at higher pH (e.g., pH 8), the interfacial films are weaker and more prone to disruption. In contrast, neutral polysaccharides, such as cellulose, exhibit minimal sensitivity to pH changes. The lower emulsion stability observed under acidic conditions suggests that the SDF fraction of SBP is less effective in stabilizing emulsions than the IDF fraction, which primarily consists of cellulose.

4. Conclusions

The influence of different modification methods on the chemical composition and fiber content of sea buckthorn pomace was evaluated. Supercritical CO_2 extraction, used to separate the lipophilic fraction, resulted in an 85.7% reduction in fat content, along with a 20.1% increase in protein content and a 10% increase in total fiber content. Enzymatic hydrolysis decreased both soluble and insoluble fiber content, but led to an increase in mono- and oligosaccharides, as well as free phenolic compounds. Among the tested enzymes, Viscozyme® L (with pectinolytic and cellulolytic activity) was most effective in hydrolyzing soluble fibers, while Celluclast® 1.5 L (with cellulolytic activity) was most efficient in breaking down insoluble fibers. Enzymatic treatment also improved the swelling capacity, water and oil retention, and solubility of the pomace; however, it had not a significant impact on the emulsions' stabilizing properties.

These findings support the valorization of sea buckthorn pomace as a valuable source of dietary fibers for the development of functional food ingredients. The application of targeted modification techniques can enhance the nutritional and most technological properties of pomace-derived fibers, although no improvement in emulsion stability was observed. These modifications contribute to waste reduction and the promotion of human health. This aligns with current trends in sustainable food production and the circular bioeconomy, encouraging the efficient use of plant-based by-products.

Author Contributions: Conceptualization, L.B. and D.Č.; methodology, J.J., M.S. and D.Č.; software, J.J. and G.K.; validation, J.J.; G.K. and M.S.; formal analysis, J.J., G.K., M.S., D.Č. and L.B.; investigation, G.K. and M.S.; resources, L.B.; data curation, L.B. and D.Č.; writing—original draft preparation, G.K., J.J. and D.Č.; writing—review and editing, L.B., D.Č., J.J. and M.S.; visualization, J.J. and D.Č.; supervision, L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: The authors acknowledge Novozymes A/S, Denmark, for kindly providing the enzymes used in this research.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BD	bulk density
TDF	total dietary fiber
TPC	total phenolic content
SDS	soluble dietary fiber
IDF	insoluble dietary fiber
ORC	oil retention capacity
WSI	water solubility index
WRC	water retention capacity
WSC	water swelling capacity
SBP	sea buckthorn pomace
SBP-CO_2	sea buckthorn pomace modified using supercritical carbon dioxide extraction
SFE-CO_2	supercritical carbon dioxide extraction

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