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# Composition and Technological Properties of Modified Lingonberry (*Vaccinium vitis-idaea* L.) Pomace

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**Abstract:** Lingonberry pomace (LP) is a by-product rich in valuable bioactive compounds and can be used in the food industry after various treatments and property characterization. This study aimed to evaluate the impact of commercially available enzymes (Viscozyme® L, Pectinex® Ultra Tropical, and Celluclast® 1.5 L) and supercritical carbon dioxide (SFE-$CO_2$) extraction technology on the chemical composition and technological properties of treated LP products. The Megazyme kit was used to determine the soluble dietary fiber (SDS) and insoluble dietary fiber (IDF) contents, while the changes in mono-, disaccharide, and oligosaccharides were analyzed by applying high-pressure liquid chromatography with a refractive index detector. The analyzed properties were as follows: the water swelling capacity (WSC), water retention capacity (WRC), water solubility index (WSI), oil retention capacity (ORC), bulk density (BD), and emulsion stability of modified LP. The tested LP contained 8.49 g/100 g of SDF and 65.36 g/100 g of IDF (in dry matter). The partial separation of lipophilic substances during SFE-$CO_2$ extraction did not significantly affect the enzymatic hydrolysis efficiency. The amount of oligosaccharides in the LP increased using enzymes with pectinolytic activity (Viscozyme® L and Pectinex® Ultra Tropical), while cellulolytic enzymes (Celluclast® 1.5 L) increased the amount of SDF and improved the IDF/SDF ratio. Enzymatic hydrolysis increased the SI, WRC, and ORC of LP powder. Emulsions with LP hydrolyzed with Pectinex® Ultra Tropical demonstrated the highest stability during storage. This study demonstrates that the modification of LP powders provides diverse technological properties, which could expand the application of such products for further food production.

**Keywords:** lingonberry pomace; enzymatic hydrolysis; technological properties; dietary fiber; oligosaccharides



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## 1. Introduction

Lingonberry (*Vaccinium vitis-idaea*) is a wild plant of the *Ericaceae* family, widely grown throughout the Northern Hemisphere from Asia and Europe to North America, with small red berries, which are traditionally used as food and as a medicine due to their health benefits [1]. These berries contain high levels of vitamins C, A, and E and phenolic compounds with high antioxidant properties [2]. However, one of the obstacles that limit the use of lingonberries is their characteristic intense sour or even bitter taste; therefore, these berries are usually used in the production of jams and juices [3,4]. After juice processing, a considerable amount of high-added-value by-products such as dietary fiber, polyphenolic compounds, antioxidants, pigments, essential minerals, fatty acids, and others remain in the pomace [5]. The generation of food waste is a critical problem from economic

and environmental points of view. Therefore, sustainable product development based on circular economy principles like by-product processing in food products is relevant and attracts the interest of scientists. The by-products of berry juicing, otherwise known as pomace, consisting of pulp, skins, seeds, and twigs, are promising sources of dietary fiber that can be used as a value-added ingredient in functional foods [6,7]. While data show that the dietary fiber of other berries such as blackcurrant and redcurrant, bilberry, chokeberry, rowanberry, or cranberry could potentially be an ingredient improving the technological and sensory properties of food products [8,9], little is known about the chemical composition of lingonberry pomace (LP), especially its dietary fiber and the technological properties. Until now, most studies have focused on phenolic compounds, organic acids, volatile organic compounds, and saccharides in lingonberry [3,10]. The recommended insoluble/soluble dietary fiber ratio for health benefits should be between 1:1 and 2:1 to achieve maximum health benefits [6]. However, such a value is rarely determined in unprocessed berry pomace; therefore, they have low functionality, which limits their use [11]. In order to improve the functionality and technological properties of these products, enzymatic, thermal, mechanical, and other processing methods are applied [12]. Processed LP could be used to produce baked goods, various snacks, milk, and meat products as an ingredient to improve functional and technological properties. In order to increase the value of LP and the possibilities of its application, a detailed assessment of the composition and technological properties is necessary.

Supercritical carbon dioxide (SFE-$CO_2$) extraction, which separates essential oils and lipophilic and polyphenolic compounds, has recently attracted much scientific interest [13,14]. Applying this technology reduces the lipophilic content, which may have an effect on the technological properties. Moreover, enzyme application may modify the saccharide composition and technological properties of berry pomace [9]. However, there are a lack of data on the influence of various processing methods on LP's composition and technological properties.

This study aimed to evaluate the influence of commercially available enzymes and CFE-$CO_2$ extraction technology on the modification possibilities of LP fiber by increasing the SDF content and evaluate the chemical composition and technological properties of the modified products.

## 2. Materials and Methods

### *2.1. Lingonberry Pomace Characteristics and Enzymatic Modification*

Fresh LP was kindly donated by a local company JSC 'FUDO' (Kaunas, Lithuania). After juice pressing, the pomace (~15.3% of the fresh berry mass) was dried at 35–40 °C for 48 h to ~3.4% residual moisture content and ground using a ZM 200 mill (Retsch, Haan, Germany) to a particle size of less than 500 µm. The modification of LP was carried out using SFE-$CO_2$ extraction in a Helix extraction system (Applied Separation, Allentown, PA, USA) at the following parameters: pressure, 45 MPa; temperature, 50 °C; extraction time, 240 min; $CO_2$ flow, 2 SL/min. Obtained modified LP after extraction (LP-$CO_2$) was used for further analysis. The rationale behind the applied extraction conditions relied on a previous publication that optimized SFE in lingonberry pomace reported by Kitrytė et al. [15].

For LP enzymatic modification, Novozymes A/S (Bagsvaerd, Denmark) commercial enzymes such as Viscozyme® L (100 FBG/g), Pectinex® Ultra Tropical (5000 PECTU/g), and Celluclast® 1.5 L (700 EGU/g) were used. An amount of 2.5 g of sample was mixed with 37.5 mL of distilled water and 0.25 mL of enzyme (control samples were prepared using the same amount of distilled water instead of enzyme). Hydrolysis was carried out at 50 °C for 1 h in a water bath under shaking at 200 rpm (pH 3.2; pH was not additionally adjusted). The enzymes were inactivated by heating at 90 °C for 20 min and cooled at room

temperature (20 °C). For the mono-, disaccharide, and oligosaccharide analysis, the cooled mixture after hydrolysis was centrifugated at 8000 rpm for 20 min, and the liquid part (water-soluble fraction) was collected and freeze-dried (Harvest Right, North Salt Lake, UT, USA). For the determination of the SDF and IDF and functional properties, after hydrolysis, the whole mixture was collected and freeze-dried. Freeze-dried LP samples were stored in closed, impenetrable containers at 4 °C until further analysis. Experimental schema is presented in Figure 1.

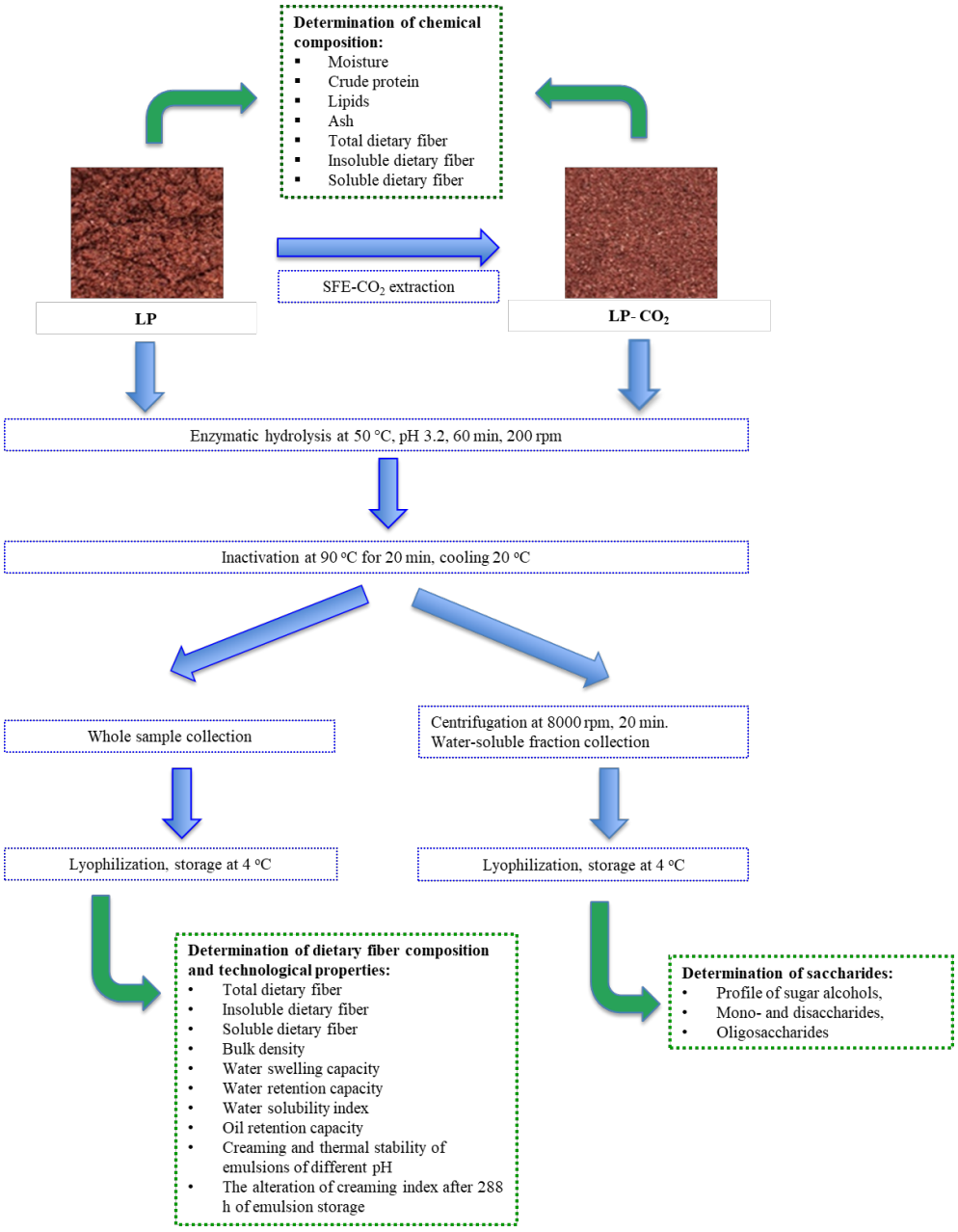


**Figure 1.** Experimental schema.

### *2.2. Chemical Composition Determination*

Moisture content was determined by drying 1 g of sample at 105 °C to constant weight based on the Association of Official Analytical Chemists (AOAC) Method 925.10-1925. The content of lipids was evaluated using Soxhlet extraction with hexane for 3 h (3 g of sample) based on AOAC 948.22. The content of crude proteins was evaluated using 1 g of sample using the Kjeldahl method (N × 6.25) based on AOAC 978.04. Ash content was

analyzed by charring 2 g of sample for 30 min and incinerating it in a muffle furnace at 625 °C for 2 h based on AOAC 930.05. Total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) were determined using a Megazyme's Total Dietary Fiber assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) as described by the manufacturer [16]. Am amount of 1 g of sample was mixed with MES-Tris buffer (40 mL, 0.05 M pH 8.2) and hydrolyzed with α-amylase (50 µL, 30 min, 95 °C) and protease (100 µL, 30 min, 60 °C) under stirring at 120 rpm. After hydrolysis, the pH was adjusted to 4.1–4.8 with 0.56 1 N HCl, and further hydrolysis was performed with amyloglucosidase (200 µL, 30 min, 60 °C) under stirring at 120 rpm. The used enzymes were provided in Megazyme's Total Dietary Fiber assay kit. The hydrolysate was filtered (Fibertec 1023 E, Foss System, Hilleroed, Denmark), and the residue was washed with 10 mL of hot distilled water (70 °C), 10 mL of 95% ethanol, and acetone; dried overnight in a 103 °C oven; and weighed and recorded as IDF. For SDF evaluation, liquid after filtration and combined solutions after washing were mixed with 4 volumes of 95% ethanol, stored for 1 h at room temperature and filtered. The obtained residue was washed with 78 and 95% ethanol and acetone. After washing, the residue was dried and weighed. Protein and ash contents were determined in dried IDF and SDP and subtracted. The sum of IDF and SDF was taken as TDF.

### *2.3. Saccharide Analysis by High-Pressure Liquid Chromatography with Refractive Index Detector*

For the saccharide analysis, 10 mg of the freeze-dried water-soluble fraction was dissolved in 1 mL of Millipore water (10 mg/mL). Mono- and disaccharide analyses were performed according to Syrpas et al. [17] using a Thermo Scientific Ultimate 3000 HPLC system coupled to a RefractoMax 521 refractive index detector (Thermo Fisher Scientific, Waltham, MA, USA). Saccharide components were separated using two sugar columns in series, SUGAR KS-802 and KS-801 (8.0 mmID × 300 mm each), with ultrapure water as a mobile phase. The columns were operated at 80 °C with an isocratic flow rate of 0.5 mL/min. Samples were run for 60 min, and the injection volume was 10 µL. Chromatograms were recorded and processed using Chromeleon 7 software (Thermo Fisher Scientific, Waltham, MA, USA).

### *2.4. The Analysis of Technological Properties*

The water swelling capacity (WSC) of lingonberry samples was analyzed as described by Yu et al. [18] with some modifications. In a centrifuge tube, −0.200 g of sample was added, and the occupied volume was recorded. The sample was mixed with distilled water (6 mL), covered, and stored at 21 °C. After 18 h of storage, the increased volume was recorded. WSC was calculated using Formula (1):

$$\text{WSC (mL/g)} = (V1 - V0)/M \quad (1)$$

where V0 is the volume of the dry sample (mL); V1 is the volume of the sample after hydration (mL); and M is a mass of the sample recalculated in dry matter (g).

The water retention capacity (WRC) of lingonberry samples was performed according to Yu et al. [18] with some modifications. For WRC determination, the same sample used for WSC analysis was centrifuged at 5000 rpm for 20 min, decanted, weighed, dried at 105 °C and weighed after drying. The WRC was calculated using Formula (2):

$$\text{WRC (g/g)} = (M1 - M2)/M2 \quad (2)$$

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

A supernatant was used after decantation for water solubility index (WSI) determination. The supernatant was dried at 105 ± 2 °C until a constant weight was reached, and the WSI was calculated using Formula (3):

$$\text{WSI (\%)} = (M/M0) \times 100 \quad (3)$$

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

The oil retention capacity (ORC) of lingonberry samples was evaluated according to Yu et al. [18] with some modifications. For ORC, 0.2 g of sample was mixed with 2 g of sunflower oil, stored at 21 °C for 1 h, and centrifuged at 3000× *g* (Microcen 23, Ortoalresa, Madrid, Spain) for 10 min. Oil was decanted, and the pellets were weighted. The ORC was calculated using Formula (4):

$$\text{ORC (g/g)} = (W1 - W0)/W0 \quad (4)$$

where W1 is the weight of the pellets (g); W0 is the weight of the dry sample in dry matter (g).

The lingonberry samples' bulk density (BD) was evaluated according to Jagelavičiūtė et al. [19]. An amount of 0.200 ± 0.001 g of sample was weighed in a graduated test tube. The test tube was gently shaken by tapping it on the table's surface 20 times, and the volume occupied by the sample was measured. The BD was calculated according to Formula (5):

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

where M is the weight of the sample in dry matter (g); V is the sample volume occupied in the test tube (mL).

### 2.5. Emulsion Preparation and Analysis of Stability

For emulsion preparation, 0.16 g of sample was mixed with 8 mL of distilled water and 8 g of sunflower oil in graduated tubes. Emulsification was performed using a homogenizer (IKA® T-25 digital, Ultra-Turrax, Staufen, Germany) for 5 min at 10,000 rpm. The stability of the emulsions was evaluated under creaming (static) and by evaluating creaming index (CI). For creaming stability evaluation, after 30 min of storage at room temperature, CI was calculated according to Keršienė et al. [20] using Formula (6). For thermal stability evaluation, emulsified samples were heated in water bath at 80 °C for 30 min and CI was calculated according to Formula (6). Additionally, emulsion stability was evaluated after 12 days of storage at 4 °C, and the alteration in CI after 288 h of emulsion was expressed in percent that a value was altered. For pH effect evaluation, instead of water, buffer solutions of 0.1 M citric acid and 0.2 M $Na_2HPO_4$ were used to obtain final pH values of 4.0, 6.0, and 8.0.

$$\text{Creaming index (\%)} = (SV/TV) \times 100 \quad (6)$$

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

### 2.6. Statistical Analyses

Experiments were carried out in triplicate. The mean values and standard deviations were calculated using MS Excel 2019 (Microsoft Corp, Albuquerque, NM, USA). The statistical analysis was performed using the statistical package Statgraphics Centurion 19. One-way analysis of variation (ANOVA), followed by Tukey's honest significant difference (HSD) test, was carried out to evaluate significant differences ($p \leq 0.05$).

## 3. Results

### 3.1. Chemical Composition of LP and LP-$CO_2$

The influence of SFE-$CO_2$ on the chemical composition of the LP before and after extraction is shown in Table 1. The LP's main constituent is TDF, followed by lipids and proteins. SFE-$CO_2$ extraction reduced the lipid content by 40% and increased the crude protein, ash, SDF, IDF, and mono- and oligosaccharide contents by 5.0, 5.1, 4.4, 5.3, and 34.0%, respectively. Previous studies indicated that berry cell walls' main dietary fiber compounds are lignin, cellulose, hemicellulose, and pectin, which involve homogalacturonan and rhamnogalacturonan II [21]. The dietary fiber in berry pomaces is usually higher than 20% and depends on berry type, processing conditions, cultivar, and ripeness. The SDF/IDF ratio influences the technological applications of berry pomace in foods; moreover, from a nutritional point of view, this ratio should be between 1:1 and 2:1 to achieve maximum health benefits [6]. The SDF/IDF ratios in LP and LP-$CO_2$ were 7.7 and 7.6, respectively. Therefore, additional solutions, such as modifications using enzymatic hydrolysis, are needed to increase SDF and improve this ratio.

**Table 1.** Chemical composition of LP before and after SFE-$CO_2$ extraction (LP-$CO_2$), g/100 g DM.

| Parameter | LP | LP-$CO_2$ |
|---|---|---|
| Moisture, % | 3.41 ± 0.04 $^{a}$ | 3.58 ± 0.09 $^{b}$ |
| Crude protein | 8.60 ± 0.28 $^{a}$ | 9.03 ± 0.01 $^{b}$ |
| Lipids | 12.68 ± 0.39 $^{a}$ | 7.60 ± 0.04 $^{b}$ |
| Ash | 1.18 ± 0.01 $^{a}$ | 1.24 ± 0.01 $^{b}$ |
| TDF | 73.85 ± 0.79 $^{a}$ | 77.20 ± 1.62 $^{b}$ |
| IDF | 65.36 ± 0.69 $^{a}$ | 68.26 ± 1.47 $^{b}$ |
| SDF | 8.49 ± 0.1 $^{a}$ | 8.94 ± 0.15 $^{b}$ |
| IDF/SDF ratio | 7.698 | 7.635 |
| $^{1}$ Mono- and oligosaccharides | 3.68 ± 1.22 $^{a}$ | 4.93 $^{a}$ ± 1.20 $^{b}$ |

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

### 3.2. Chemical Composition of Enzymatically Treated LP

Enzyme preparations used for carbohydrate hydrolysis were selected based on research by other scientists. Nguyen et al. [22] found that treating berry pulp with Viscozyme® L and Pectinex® Ultra SP significantly increased the extraction yield and total soluble solids compared to juice pressing without enzymes. Wikiera'a et al. [23] found that processing the pomace with Celluclast® 1.5 L resulted in the highest pectin yield among tested enzymatic preparations. The research findings suggest that this enzymatic preparation has a broad action profile capable of liberating pectins from the cell walls of plants rich in cellulose and hemicellulose.

All used enzyme preparations reduced ($p < 0.05$) the TDF content in LP and LP-$CO_2$ (Table 2) compared to the enzymatically untreated samples (Table 1). The highest reduction in TDF was reached using Viscozyme® L and Pectinex® Ultra Tropical (19.8 and 18.9% in LP and 18.9 and 17.3% in LP-$CO_2$, respectively). A smaller reduction in TDF was found using Celluclast® 1.5 L for LP and LP-$CO_2$ modifications (6.8 and 6.6%, respectively). SDF was hydrolyzed at a higher degree compared with IDF using Viscozyme® L and Pectinex® Ultra Tropical. The reduction in SDF reached 53.1 and 53.6% in LP, while it reached 54.6 and 49.29% in LP-$CO_2$, respectively. A high reduction in SDF had an adverse effect on the IDF/SDF ratio in the modified LP products. The results show that smaller fragments of hydrolysis can be obtained which cannot be precipitated with ethanol. Opposite trends were found when Celluclast® 1.5 L was used for LP enzymatic treatment. This enzyme preparation increased ($p < 0.05$) the content of SDF in LP by 13% and in LP-$CO_2$ by 11.6%

compared to the untreated raw material, and the IDF/SDF ratio for LP was improved from 7.6 to 6.1, while that for LP-$CO_2$ improved from 7.6 to 6.2; however, this ratio did not reach the recommended value. The IDF content was reduced more effectively using Viscozyme® L and Pectinex® Ultra Tropical (15.5 and 14.4% in LP and 14.3 and 13.1% in LP-$CO_2$, respectively) compared with Celluclast® 1.5 L (9.4 and 9.0% in LP and LP-$CO_2$, respectively). The partial separation of lipophilic substances during SFE-$CO_2$ extraction did not have a statistically significant effect on the enzymatic hydrolysis efficiency. There is a lack of data in the literature on the modification of LP with enzymes or other pre-treatments with the aim to increase the amount of SDF, while cereal and legume by-products with possibilities of increasing the SDF content are widely studied through applying not only enzymatic treatments, but also physical treatments, such as high hydrostatic pressures and thermal and extrusion techniques [24]. Treatment with high hydrostatic pressures increases the SDF content in mango and orange peel by 15 and 95%, respectively, while applying extrusion treatment for garlic skin and wheat bran increased the SDF content by 199 and 98%, respectively [24]. Gu et al. [25] reported that enzymatic modification using Viscosyme L could enhance the extraction yield of SDF in tomato peels by up to 72%. Mrabet et al. [26] reported that SDF increased by three times using Viscozyme® L treatment at optimized conditions in date fruit samples. Yoon et al. [27] applied cellulase-rich crude enzyme preparation previously isolated from edible snails that increased the alcohol SDF in carrot pomace.

**Table 2.** Effect of enzymatic treatment on dietary fiber composition of LP products, g/100 g DM.

| LP Modification | LP-V | LP-PU | LP-C | LP-$CO_2$-V | LP-$CO_2$-PU | LP-$CO_2$-C |
|---|---|---|---|---|---|---|
| TDF | 59.21 ± 0.31 $^{a}$ | 59.9 ± 1.0 $^{a}$ | 68.82 ± 0.05 $^{c}$ | 62.6 ± 0.12 $^{b}$ | 63.86 ± 0.55 $^{b}$ | 72.1 ± 0.09 $^{d}$ |
| IDF | 55.22 ± 0.38 $^{a}$ | 55.97 ± 0.97 $^{ab}$ | 59.21 ± 0.26 $^{c}$ | 58.49 ± 0.18 $^{bc}$ | 59.33 ± 0.42 $^{c}$ | 62.12 ± 0.12 $^{d}$ |
| SDF | 3.99 ± 0.07 $^{a}$ | 3.93 ± 0.03 $^{a}$ | 9.62 ± 0.21 $^{c}$ | 4.11 ± 0.06 $^{a}$ | 4.53 ± 0.13 $^{b}$ | 9.98 ± 0.03 $^{c}$ |
| IDF/SDF ratio | 13.840 $^{b}$ | 14.242 $^{b}$ | 6.155 $^{a}$ | 14.231 $^{b}$ | 13.097 $^{c}$ | 6.224 $^{a}$ |

Mean ± standard deviation values in row with different lowercase letters are significantly different ($p < 0.05$). TDF—total dietary fiber; IDF—insoluble dietary fiber; SDF—soluble dietary fiber; LP—lingonberry pomace; LP-V—lingonberry pomace after hydrolysis with Viscozyme® L; LP-PU—lingonberry pomace after hydrolysis with Pectinex® Ultra Tropical; LP-C—lingonberry pomace after hydrolysis with Celluclast® 1.5 L; LP-$CO_2$—lingonberry pomace after $CO_2$ extraction; LP-$CO_2$-V—lingonberry pomace after $CO_2$ extraction and hydrolysis with Viscozyme® L; LP-$CO_2$-PU—lingonberry pomace after $CO_2$ extraction and hydrolysis with Pectinex® Ultra Tropical; LP-$CO_2$-C—lingonberry pomace after $CO_2$ extraction and hydrolysis with Celluclast® 1.5 L.

The Viscozyme® L enzyme complex used for hydrolysis is composed of various carbohydrases, including polygalacturonases (pectinases), $\beta$-glucanase, arabinase, cellulase, hemicellulases, and xylanases, while Pectinex® Ultra Tropical contains pectinlyases, polygalacturonases, $\beta$-glucanase, and cellulase. The predominant polysaccharides in fruit and berry cell walls are known to be cellulose, which accounts for 30%; hemicelluloses, which account for 30%; and pectins, which account for 35%, with the remainder of the wall being composed of proteins at 5% [28]. The fiber content was the least reduced after the treatment of LP with the Celluclast® 1.5 L enzyme preparation in which the main enzymes are cellulase and $\beta$-glucanase. Using enzymes like cellulases, pectinases, or hemicellulases can break down cell walls and increase the release of soluble fiber from berry pomace. This helps release pectin (soluble fiber) and other polysaccharides present in the pomace.

### 3.3. Technological Properties of Modified LP

Technological property evaluation is important for the effective incorporation of pomace into food products as a food ingredient as it contributes to the textural attributes and stability of formulated foods. Various sources in the literature claim that the interaction

of pomace with water and oil is determined by the particle size, bulk density, porosity, and surface area [29,30]. After the enzymatic hydrolysis of LP and LP-$CO_2$, the BD decreased by 10–23% and 13–23%, respectively ($p < 0.05$). It was noticed that LP and LP-$CO_2$ hydrolyzed with Celluclast® 1.5 L had the highest SDF content and the lowest BD (Table 3). The decrease in BD can be attributed to the more efficient hydrolysis of DF by reducing the particle size and forming compounds with a lower molecular weight.

**Table 3.** Technological properties of modified LP.

| | BD, g/mL | WSC, mL/g | WRC, g/g | WSI, % | ORC, g/g |
|---|---|---|---|---|---|
| LP | 0.30 ± 0 $^{d}$ | 1.298 ± 0.004 $^{c}$ | 5.03 ± 0.20 $^{a}$ | 19.20 ± 0.05 $^{b}$ | 2.27 ± 0.02 $^{a}$ |
| LP-$CO_2$ | 0.30 ± 0.001 $^{d}$ | 1.296 ± 0.006 $^{c}$ | 4.88 ± 0.09 $^{a}$ | 18.62 ± 0.05 $^{a}$ | 2.30 ± 0.01 $^{a}$ |
| LP-V | 0.26 ± 0.001 $^{c}$ | 1.075 ± 0.005 $^{b}$ | 6.08 ± 0.11 $^{d}$ | 23.33 ± 0.33 $^{e}$ | 3.27 ± 0.03 $^{b}$ |
| LP-PU | 0.25 ± 0.001 $^{b}$ | 1.073 ± 0.004 $^{b}$ | 6.12 ± 0.01 $^{d}$ | 26.28 ± 0.08 $^{g}$ | 3.35 ± 0.05 $^{b}$ |
| LP-C | 0.23 ± 0.001 $^{a}$ | 0.538 ± 0.003 $^{a}$ | 5.68 ± 0.01 $^{c}$ | 22.53 ± 0.06 $^{d}$ | 3.75 ± 0.02 $^{c}$ |
| LP-$CO_2$-V | 0.26 ± 0 $^{c}$ | 1.078 ± 0 $^{b}$ | 5.91 ± 0.01 $^{d}$ | 23.26 ± 0.01 $^{e}$ | 3.34 ± 0.01 $^{b}$ |
| LP-$CO_2$-PU | 0.25 ± 0.001 $^{b}$ | 1.075 ± 0.003 $^{b}$ | 5.61 ± 0.02 $^{c}$ | 24.47 ± 0.21 $^{f}$ | 3.69 ± 0.01 $^{c}$ |
| LP-$CO_2$-C | 0.23 ± 0 $^{a}$ | 0.537 ± 0 $^{a}$ | 5.35 ± 0.01 $^{b}$ | 19.78 ± 0 $^{c}$ | 3.86 ± 0.05 $^{d}$ |

Mean ± standard deviation values in column with different lowercase letters are significantly different ($p < 0.05$). BD—bulk density; WSC—water swelling capacity; WRC—water retention capacity; WSI—water solubility index; ORC—oil retention capacity; LP—lingonberry pomace; LP-V—lingonberry pomace after hydrolysis with Viscozyme® L; LP-PU—lingonberry pomace after hydrolysis with Pectinex® Ultra Tropical; LP-C—lingonberry pomace after hydrolysis with Celluclast® 1.5 L; LP-$CO_2$—lingonberry pomace after $CO_2$ extraction; LP-$CO_2$-V—lingonberry pomace after $CO_2$ extraction and hydrolysis with Viscozyme® L; LP-$CO_2$-PU—lingonberry pomace after $CO_2$ extraction and hydrolysis with Pectinex® Ultra Tropical; LP-$CO_2$-C—lingonberry pomace after $CO_2$ extraction and hydrolysis with Celluclast® 1.5 L.

Enzymatic modification of the LP reduced the WSC. The lowest WSC was determined in LP and LP-$CO_2$ hydrolyzed with Celluclast® 1.5 L, which was lower by 58% in both cases ($p < 0.05$) compared to the untreated LP. It was noticed that the LP and LP-$CO_2$ hydrolyzed with Celluclast® 1.5 L had the lowest BD, which could lead to a lower WSC. Previous studies on swelling capacity reported higher values for lingonberry pomace (7.90 and 8.00 mL/g at pH 2 and 7, respectively) [31]. Čechovičienė et al. [32] reported WSC values of different blackberry cultivars in the range of 1.13–2.27 mL/g, which are values close to our findings.

Enzymatic hydrolysis increased the WRC of LP and LP-$CO_2$ in all cases. Enzyme treatment increased the WRC of LP by 13–22% and that of LP-$CO_2$ by 10–21%. The increase in the WRC after enzymatic modification could be due to the increase in SDF after treatment with Celluclast® 1.5 L and the increase in oligosaccharides after treatment with Viscozyme® L and Pectinex® Ultra Tropical (Section 3.4). A lower water binding capacity (2.78–4.32 g/g) was reported by Jurevičiūtė et al. [30] in berry pomace at pH 2 and 7, while higher values (10.59 g/g) were reported by Jagelavičiūtė et al. [9] in cranberry pomace.

According to Reibner et al. [33], a larger surface area of particles positively affects the WRC and WSC; on the contrary, a smaller surface area leads to a lower WRC. A lower WRC may also be obtained due to a lower carbohydrate content. Witczak et al. [34] found that sugar-enriched orange peels absorbed up to 60% more water than normal peels. Nemetz et al. [35] stated that the ability of chokeberry, bilberry, and elderberry pomace to retain water depended on their bulk density. The research results obtained during this study confirm this statement. LP powders with lower BD had a better WRC and a lower WSC.

Enzymatic hydrolysis increased the WSI of LP (by 20–37%) and LP-$CO_2$ (6–31%) in all cases. Nemetz et al. [35] concluded that a high amount of insoluble polysaccharides caused the low solubility of pomace powder in water, affecting their hydrophobicity and electrostatic properties, which led to the formation of aggregates and a decrease in solubility. The higher WSI of the cellulolytic enzyme-treated LP was due to increases in the SDF and

mono- and oligosaccharides and a decrease in the IDF. LP and LP-$CO_2$ hydrolyzed by pectinolytic enzymes showed higher solubility; in these samples, a higher amount of oligosaccharides was formed than those treated with cellulolytic enzymes (Section 3.4).

In all cases, the enzymatic modification of LP and LP-$CO_2$ increased ($p < 0.05$) the ORC by 41–65% and 45–68%, respectively. The ORC is a functional property of berry dietary fiber used in food manufacturing, while ingredients with a high ORC can be used as emulsion stabilizers. Previous studies demonstrated a lower ORC for lingonberry, pear, cranberry, and blueberry pomace (1.46 g/g [31], 1.82 g/g [36], 1.97 g/g, and 1.96 g/g [37], respectively) and a higher ORC for red raspberry pomace (2.44 g/g [38]) compared with LP.

LP and enzymatically modified LP products were used for emulsion preparation. The effect of temperature and different pH values were studied to evaluate emulsion stability under stresses (Figure 2). Thermally untreated emulsions were more stable. Enzymatically modified LP showed better emulsion stabilizing properties after the heat treatment (30 min at 80 °C) of emulsions compared to the untreated LP at the tested pH range. The same tendency was found for the LP-$CO_2$ samples. The highest creaming stability (the lowest CI) of all emulsions was seen at pH 6. The emulsions' lowest thermal stability (the highest CI) was seen at pH 4 (except for LP-$CO_2$-PU). Among the heat-treated emulsions, LP and LP-$CO_2$ hydrolyzed with Pectinex® Ultra Tropical had the highest thermal stability at pH 4, while at pH 6, the highest stability was demonstrated for LP and LP-$CO_2$ hydrolyzed with Celluclast® 1.5 L.

To evaluate the effect of different LP products and pH on the emulsion's stability during storage, the CI alteration after 288 h of storage at +4 °C was calculated (Figure 3). The emulsions with enzymatically untreated LP and LP-$CO_2$ were more stable during storage at pH 6 under static (creaming) conditions (Figure 3A,B), while after heat treatment they were more stable at pH 4 (Figure 3C,D).

During storage, LP hydrolyzed with Celluclast ® 1.5 L improved emulsion creaming stability at pH 4 and 8, while LP hydrolyzed with Pectinex® Ultra Tropical improved emulsion creaming stability at pH 4, 6, and 8, and LP hydrolyzed with Viscozyme® L improved emulsion creaming stability at pH 6 and 8 compared with the enzymatically untreated LP.

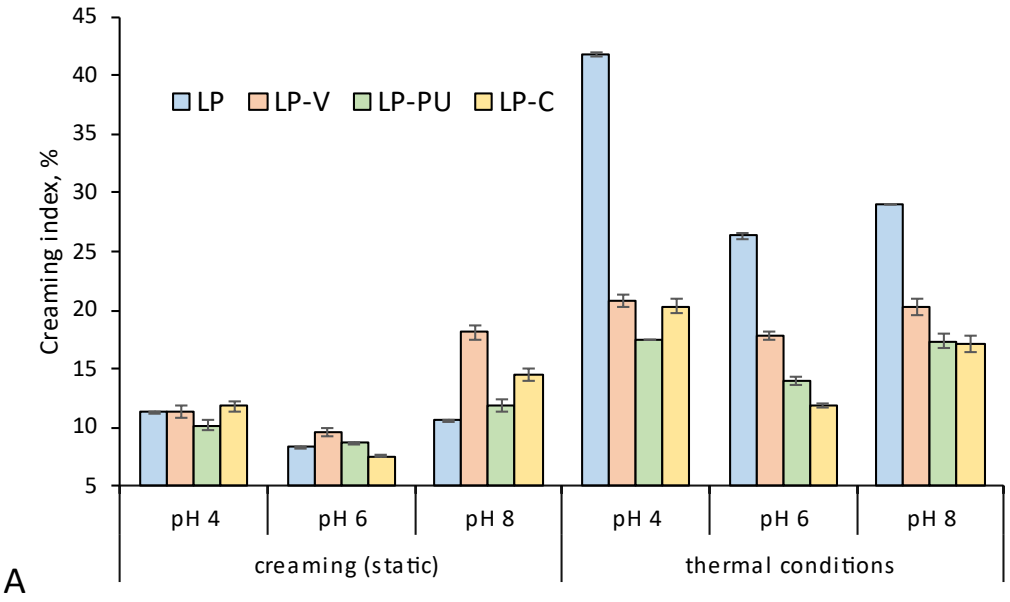


**Figure 2.** *Cont.*

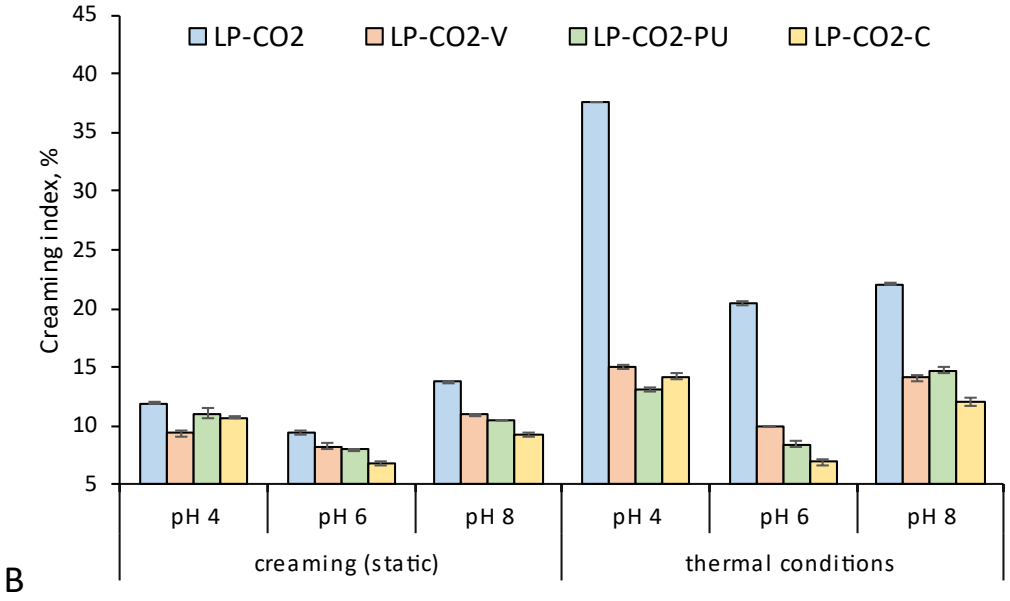


**Figure 2.** pH effect on creaming and thermal stability of emulsions prepared with enzymatically modified LP products (**A**) and LP obtained after SFE-$CO_2$ extraction and enzyme modifications (**B**). V—Viscozyme® L; PU—Pectinex® Ultra Tropical; C—Celluclast® 1.5 L.

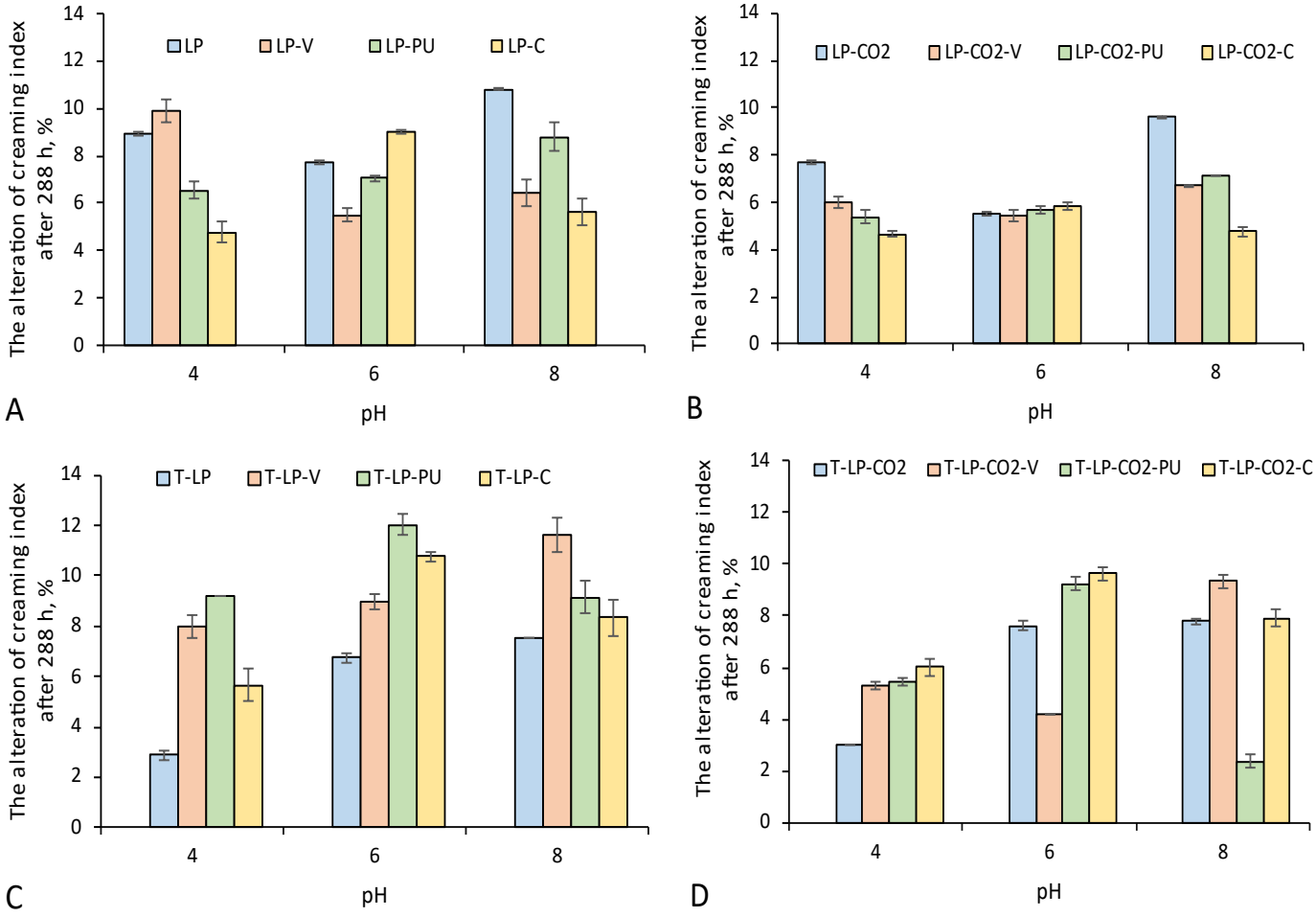


**Figure 3.** pH effect on changes in creaming (**A**,**B**) and thermal (**C**,**D**) stability of emulsions prepared with enzymatically modified LP products (**A**,**C**) and LP obtained after $CO_2$ extraction and enzyme modifications (**B**,**D**) during storage for 288 h at 4 °C. V—Viscozyme® L; PU—Pectinex® Ultra Tropical; C—Celluclast ® 1.5 L.

According to the literature, there is no single reason for why berry pomace and its products have stabilizing properties. Two main hypotheses for the stabilization of emulsions with berry/fruit pomace powder are proposed in the literature: (i) the insoluble pomace fraction together with the soluble fraction (soluble proteins, pectins, saccharides, etc.) increases the viscosity of emulsions (emulsions with a more viscous dispersion medium are more stable); (ii) oil droplets in the emulsion are stabilized by small particles of the insoluble fraction, which adsorb on the separating surface of the droplets and prevent them from coalescing [39–41].

Mathis et al. [42] identified three main factors that determine the emulsion stabilization properties of berry/fruit pomaces: (i) the size of the pomace particles, (ii) the amount of insoluble fiber, and (iii) the WRC. In their work, apple pomace powder, which had the smallest particle size, insoluble fiber content, and water-holding capacity compared to sugar beet and oat by-products, had the best emulsion stabilization properties. The conclusions of this study state that insoluble fibrous materials (solid particles) can be used as emulsion stabilizers that change the size of oil droplets, adsorb at the oil–water interface, and prevent phase coalescence. Soluble components of pomace can also have a stabilizing effect because they can adsorb on the oil–water interface, and pectins are an additional stabilizing factor that increases the viscosity of the emulsion (forming a pectin gel). The optimal amount of saccharides is essential for forming pectin gels: stronger gels are formed at higher amounts. Heat treatment also leads to pectin solutions having a higher viscosity and the formation of pectin layers around the fat globules, preventing coalescence [43]. Moreover, soluble components can have a synergistic or antagonistic effect in the interaction with insoluble fiber materials [42].

### *3.4. Mono- and Oligosaccharides of Modified LP*

Megazyme's methodology for TDF determination does not allow for the evaluation of oligosaccharides present in the tested material which, according to the Codex Alimentarius Commission's definition, can be classified as dietary fiber and are important due to their prebiotic properties [44]. Therefore, the influence of enzymatic hydrolysis on the changes in mono- and oligosaccharides in LP was analyzed (Figure 4). Samples after SFE-$CO_2$ extraction were selected for mono- and oligosaccharide evaluation because of the higher SDF content.

The enzymatic modification of LP-$CO_2$ using Viscozyme® L and Pectinex® Ultra Tropical increased the amount of galacturonic acid (by 47 and 46%, respectively), which is the main component of pectin. Celluclast® 1.5 L increased monosaccharides (glucose and fructose) and disaccharides (sucrose). In LP-$CO_2$ samples hydrolyzed with Celluclast® 1.5 L, the glucose, fructose, and sucrose contents increased by 4, 12, and 43%, respectively, compared with the LP-$CO_2$ sample, which was not treated with enzyme preparations. Viscozyme® L and Pectinex® Ultra Tropical did not significantly affect mono- and disaccharide changes.

Enzyme preparations with pectinolytic activity, Viscozyme® L and Pectinex® Ultra Tropical, degraded the SDF (Table 2), which led to a significant increase in oligosaccharides (Figure 3). After evaluating the profile of oligosaccharides, it was observed that the mentioned enzyme preparations led to a significant increase in oligosaccharides consisting of 5-6 monosaccharide units (DP5-6). The content of these oligosaccharides increased from 0 g/mg DM to 3.2 g/mg DM and 2.8 mg/g DM in the LP-$CO_2$ samples hydrolyzed with Viscozyme® L and Pectinex® Ultra Tropical, respectively, while Celluclast® 1.5 L only resulted in an increase to 0.6 mg/g DM. This is also in accordance with the dietary fiber evaluation results, which show that Celluclast® 1.5 L, in contrast to the pectinolytic enzyme preparations, increased the amount of SDF (Table 2).

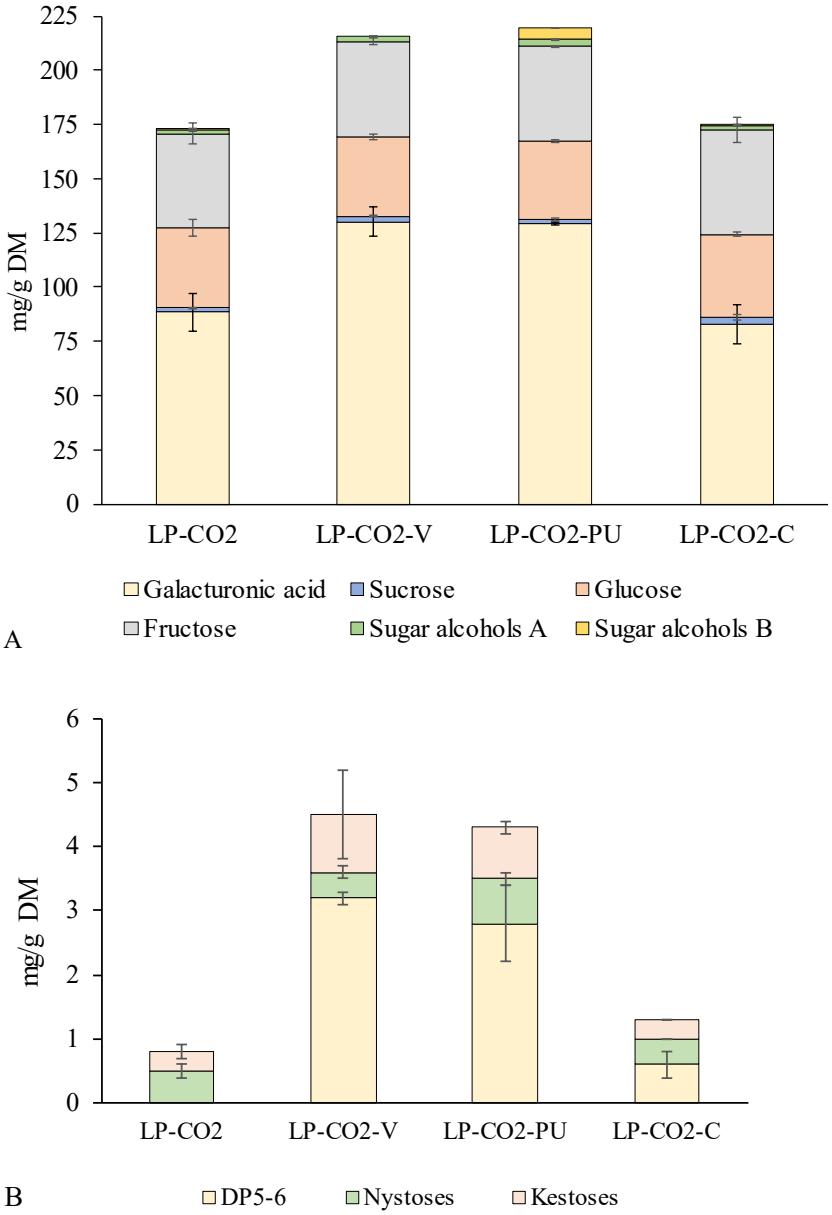


**Figure 4.** Profiles of sugar alcohols, mono- and disaccharides (**A**) and oligosaccharides (**B**) of LP after supercritical $CO_2$ extraction (LP-$CO_2$) and enzymatic hydrolysis with enzymes: V—Viscozyme® L; PU—Pectinex® Ultra Tropical; C—Celluclast ® 1.5 L. Mean ± standard deviation values (n = 3) expressed in mg/g DM.

The number of oligosaccharides, including kestoses and nystoses, did not change significantly. The results of this study suggest that LP may be a source of a new class of prebiotics called pectin oligosaccharides (POSs). POSs are indigestible oligosaccharides that selectively stimulate the growth and activity of colonic bacteria (*Bifidobacteria* and *Lactobacillus*) and positively affect human health [45]. Therefore, the enzymatic hydrolysis of pectins with Viscozyme® L and Pectinex® Ultra Tropical enzymatic preparations can increase the amount of POS in pomace, thus making it a potentially attractive ingredient for developing new functional products.

## 4. Conclusions

The powders with a particle size of <0.5 mm obtained from the lingonberry pomaces (LP) investigated in this study demonstrated good technological properties that justify their use as fiber-rich food ingredients. The enzymatic hydrolysis of LP increased its

swelling index and water and oil retention capacities. Moreover, enzymatically modified LP showed better stabilization properties of heat-treated emulsions than unmodified LP at the tested pH range. LP was observed to contain a high amount of TDF (73.85 g/100 g dry matter). The partial separation of lipophilic substances during SFE-$CO_2$ extraction reduced the lipid content by 40%, while it did not have a statistically significant effect on the enzymatic hydrolysis efficiency when pectinolytic (Viscozyme® L and Pectinex® Ultra Tropical) and cellulolytic enzymes (Celluclast® 1.5 L) were used. All enzyme preparations reduced the TDF content in LP and LP-$CO_2$. Enzyme preparations with pectinolytic activity (Viscozyme® L and Pectinex® Ultra Tropical) degraded the SDF, which led to a significant increase in oligosaccharides consisting of 5–6 monosaccharide units, which may be a source of prebiotics called pectin oligosaccharides that selectively stimulate the growth and activity of probiotic bacteria and have a positive effect on human health. Therefore, the enzymatic hydrolysis of pectins with Viscozyme® L and Pectinex® Ultra Tropical can increase the amount of pectin oligosaccharides in LP, thus making it a potentially attractive ingredient for developing new functional products. Summarizing the results of these studies, it can be stated that the amount of oligosaccharides in LP can be increased using enzymes with pectinolytic activity (Viscozyme® L and Pectinex® Ultra Tropical), while cellulolytic enzymes (Celluclast® 1.5 L) can be used to increase the amount of SDF and improve the IDF/SDF ratio. Enzyme-modified LP can be recommended to improve the thermal stability of emulsions, especially LP modified with Celluclast® 1.5 L, as it demonstrated the highest thermal stability of emulsions at pH 6. LP modified with Viscozyme® L and Pectinex® Ultra Tropical can be suggested as an additive for the production of bakery products (bread, cakes, and pastries), confectionary products (gelatine and puddings), and processed meat products (sausages) as it established the highest water retention capacity. LP modified with Celluclast® 1.5 L, especially LP after $CO_2$ extraction and hydrolysis with Celluclast® 1.5 L, showed the highest oil retention capacity and can therefore be suggested as additive in the production of meat substitutes and baked goods (doughnuts, croissants, and biscuits).

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| SFE-$CO_2$ | Supercritical carbon dioxide |
| TDF | Total dietary fiber |
| SDS | Soluble dietary fiber |
| IDF | Insoluble dietary fiber |

| | |
|---|---|
| WSC | Water swelling capacity |
| WRC | Water retention capacity |
| WSI | Solubility index |
| ORC | Oil retention capacity |
| BD | Bulk density |
| LP | Lingonberry pomace |
| LP-$CO_2$ | Lingonberry pomace modified using supercritical carbon dioxide extraction |
| LP-V | Lingonberry pomace after hydrolysis with Viscozyme® L |
| LP-PU | Lingonberry pomace after hydrolysis with Pectinex® Ultra Tropical |
| LP-C | Lingonberry pomace after hydrolysis with Celluclast® 1.5 L |
| LP-$CO_2$-V | Lingonberry pomace after $CO_2$ extraction and hydrolysis with Viscozyme® L |
| LP-$CO_2$-PU | Lingonberry pomace after $CO_2$ extraction and hydrolysis with Pectinex® Ultra Tropical |
| LP-$CO_2$-C | Lingonberry pomace after $CO_2$ extraction and hydrolysis with Celluclast® 1.5 L |

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