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Valorization of Cranberry Pomace Through Application in Probiotic Smoothies

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Abstract

With increasing interest in food products enriched with dietary fiber and probiotics, there is a growing need for functional ingredients that can serve both as fiber sources and as suitable components of probiotic foods. The aim of this study was to evaluate the applicability of enzymatically hydrolyzed cranberry pomace as a dietary fiber source in probiotic smoothies and to assess its effects on physicochemical and sensory properties, as well as probiotic viability during storage and under simulated gastrointestinal conditions. Smoothies were prepared using cranberry pomace hydrolyzed with the commercial enzymes Celluclast® 1.5 L or Viscozyme® L and were supplemented with *Bifidobacterium animalis* DSM 20105 and *Lactobacillus acidophilus* DSM 20079. Physicochemical parameters, sensory properties, probiotic viability during 28 days of storage at 4 °C, and survival during in vitro gastrointestinal digestion were assessed. Smoothies containing Viscozyme® L-treated pomace showed lower pH values and higher total titratable acidity, although acidity remained stable throughout storage. Pomace-enriched smoothies were perceived as more acidic and thicker but less sweet, whereas the formulation with Celluclast® 1.5 L-treated pomace received the most favorable scores for color, cranberry taste, acidity, and overall acceptability. Probiotic viability during storage was strain-dependent. *B. animalis* DSM 20105 remained above 6 $\log_{10}$ CFU/g after 28 days, whereas *L. acidophilus* DSM 20079 declined below this level during the first week. During simulated in vitro gastrointestinal digestion, a reduction in *B. animalis* viability was observed; however, viable counts remained above 6 $\log_{10}$ CFU/g in the control smoothie and in the formulation containing Celluclast® 1.5 L-treated pomace. Overall, the smoothie containing Celluclast® 1.5 L-treated pomace showed the most favorable overall performance under the storage and digestion conditions.

Keywords: cranberry pomace; enzymatically modified pomace; probiotics; smoothie

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1. Introduction

The valorization of agricultural and food processing by-products is consistent with current sustainability strategies and circular economy principles aimed at reducing food system waste and improving resource efficiency [1]. Agricultural and food processing activities generate large quantities of by-products, with approximately 88 million tons produced annually in Europe alone, a substantial proportion of which originates from fruit and berry processing [2]. Although these materials have traditionally been regarded as waste, they are increasingly recognized as valuable raw materials due to their high content of bioactive compounds, including dietary fiber, polyphenols, pigments, essential minerals, fatty acids, and other health-related components [3]. Consequently, increasing attention has

been directed towards the application of fruit and berry pomace as functional ingredients in food formulations; however, the direct incorporation of fiber-rich pomace often adversely affects technological and sensory properties, such as texture, color, and mouthfeel, which may limit consumer acceptance and industrial applicability [2,4].

Berry pomace, generated after juice extraction, primarily consists of skins, seeds, and structural tissues that retain a high proportion of complex polysaccharides [2]. These polysaccharides contribute to both insoluble and soluble dietary fiber fractions, including pectin, cellulose, hemicellulose, lignin, inulin, and related oligosaccharides [2,5]. Dietary fiber derived from berry pomace has been associated with a range of health benefits, such as reduced risk of cardiovascular diseases, obesity, diabetes, and gastrointestinal disorders [2,5]. Current dietary guidelines recommend a daily intake of approximately 25–35 g of dietary fiber, with soluble fiber playing an important role in metabolic health [6]. Therefore, increasing the dietary fiber content of food products through the incorporation of fruit and berry pomace may simultaneously address nutritional deficiencies and sustainability objectives.

To improve the functional performance of fiber-rich by-products in food applications, enzymatic treatment has been proposed as a promising modification strategy [4,7]. Enzyme-assisted processing can induce structural changes in dietary fiber, enhance the release of bound phenolic compounds and antioxidants, and modify technologically important properties, such as water-holding, swelling, and other hydration-related characteristics that are relevant for the formulation and stability of food products [7–10]. In addition, enzymatic depolymerization of cell wall polysaccharides may increase the proportion of soluble fiber and promote the formation of lower-molecular-weight carbohydrates, including oligosaccharides, which may further affect technological functionality and potential physiological value [5,9–12]. These changes are particularly important when modified pomace is intended for incorporation into food products, because altered fiber composition and hydration behavior may influence texture, consistency, and overall product performance [13,14]. Nevertheless, the effects of enzymatic treatment are highly dependent on the botanical origin and structural characteristics of the plant matrix. In some cases, enzymatic hydrolysis may reduce the total dietary fiber content while increasing the concentration of free monosaccharides [4].

The global smoothie market has demonstrated notable economic expansion, reaching an estimated value of USD 12.46 billion in 2023 and projected to grow to USD 23.08 billion by 2030, with a compound annual growth rate of approximately 9.3% over the forecast period. This trend reflects increasing consumer interest in health-oriented and convenient dietary products [15]. Several studies have demonstrated the potential of fruit pomace to enhance the nutritional and functional properties of smoothie-type beverages. For example, enrichment of apple juice with black and red currant pomace improved antioxidant and antidiabetic potential [16], while the addition of pomegranate pomace extract to strawberry–yoghurt smoothies enhanced sensory quality and shelf life [17]. These findings indicate that smoothies represent a particularly suitable matrix for the incorporation of fiber-rich ingredients, allowing the valorization of fruit pomace while maintaining product functionality and quality.

In parallel with fiber enrichment strategies, probiotics have gained considerable attention due to their documented health benefits, particularly in relation to gastrointestinal and immune function. Probiotics are defined as live microorganisms that confer health benefits on the host when administered in adequate amounts [18]. In the food industry, probiotic cultures are primarily represented by lactic acid bacteria belonging to the genera of *Lactobacillus* and *Bifidobacterium* [19]. However, maintaining probiotic viability in fruit-based matrices remains challenging, largely due to low pH values that may fall below

the optimal growth range of many probiotic strains [20]. This challenge is particularly relevant for berry-based products, which are characterized by inherently acidic pH profiles. In this context, enzymatically modified pomace may be particularly relevant not only as a source of dietary fiber, but also as a structurally altered ingredient that may influence carbohydrate availability, matrix properties, and probiotic stability.

Although fruit pomace has been incorporated into food products in previous studies [16,17], there is still limited information on the application of modified pomace, including enzymatically treated pomace with altered functional and technological properties, in different food systems. In particular, studies focusing on cranberry pomace are scarce, and the use of enzymatically treated cranberry pomace as an ingredient in smoothie-type products has not been sufficiently evaluated. In addition, limited information is available on the behavior and survival of probiotic bacteria in cranberry pomace-enriched smoothie formulations. Therefore, particular attention should be given to whether enzymatically treated pomace preparations differing in their characteristics are associated with differences in final product quality and probiotic viability. Addressing these aspects is important both for improving understanding of how such ingredients affect the quality and stability of the final product and for supporting the development of value-added functional beverages from berry processing by-products. Therefore, the aim of this study was to evaluate the applicability of enzymatically treated cranberry pomace as a fiber-rich ingredient in probiotic smoothie formulations. More specifically, the study assessed the effect of pomace incorporation on smoothie physicochemical and sensory properties, as well as its suitability for use in probiotic carrier systems by evaluating probiotic viability during refrigerated storage and simulated in vitro gastrointestinal digestion.

2. Materials and Methods

2.1. Enzymatically Hydrolyzed Cranberry Pomace

Cranberry pomace (CP) was kindly provided by the company "Įvairios sultys" (Kėdainiai, Lithuania). The pomace had a moisture content of 5.57% and was milled to a particle size of 0.5 mm and stored at 4 °C. Its chemical composition, expressed on a dry-weight basis, was as follows: crude protein, 7.40 ± 0.06%; lipids, 9.83 ± 0.46%; ash, 0.96 ± 0.04%; and dietary fibre, 71.66 ± 0.38%. The enzymatic hydrolysis of CP was performed as described by Jagelavičiūtė et al. [9]. For hydrolysis, CP was dispersed in distilled water at a 1:10 (*w*/*v*) ratio. Two commercial enzyme preparations were used: Viscozyme® L (100 FBG/g; Novozymes, Bagsværd, Denmark), containing β-glucanases, pectinases, hemicellulases, and xylanases, applied at an enzyme-to-substrate (E/S) ratio of 0.04 mL/g CP, and Celluclast® 1.5 L (700 EGU/g; Novozymes, Bagsværd, Denmark), containing cellulases, applied at an E/S ratio of 0.02 mL/g CP. According to previous characterization [9], Celluclast® 1.5 L-treated cranberry pomace showed 11.90 g/100 g dry matter of SDF and 60.52 g/100 g dry matter of IDF and 3.22 g/100 g dry matter of oligosaccharides, whereas Viscozyme® L-treated pomace contained 2.64 g/100 g dry matter of SDF, 56.46 g/100 g dry matter of IDF, and 7.16 g/100 g dry matter of oligosaccharides.

The hydrolysis step was performed for 1 h at 50 °C under shaking at 200 rpm. Following enzymatic treatment, the samples were transferred to a water bath and heated at 95 °C for 20 min to stop enzyme activity, after which they were allowed to cool to room temperature.

2.2. Preparation of Bacterial Suspensions

Lactobacillus acidophilus DSM 20079 and *Bifidobacterium animalis* DSM 20105 were propagated in De Man, Rogosa and Sharpe (MRS) broth (Biolife, Milan, Italy) supplemented with 0.05% (*w*/*v*) L-cysteine and incubated anaerobically at 37 °C for 18 h. After incubation,

the biomass was separated by centrifugation at 5000× *g* for 10 min at 4 °C. The recovered cells were washed twice with sterile 0.9% NaCl, with an additional centrifugation step performed after each wash under the same conditions. The resulting pellet was then suspended in sterile 0.9% NaCl to obtain a final cell concentration of 10.2 $\log_{10}$ CFU/mL. The prepared bacterial suspensions were subsequently used for smoothie inoculation.

2.3. Smoothies Preparation and Storage

Smoothies were formulated using enzymatically hydrolysed CP treated with either Viscozyme® L (SCPV) or Celluclast® 1.5 L (SCPC). A control smoothie without cranberry pomace (SC) was used as a reference matrix for evaluating the effect of hydrolysed pomace incorporation.

For SCPV and SCPC formulations, smoothies contained 5 g of hydrolysed CP (dry matter basis), 50 g of water, 35 g of apple juice, and 10 g of banana. After enzymatic hydrolysis, no solid–liquid separation was performed, and the resulting hydrolysate was used in its entirety for smoothie preparation. In the control formulation, pomace was omitted and replaced by water, resulting in a composition of 55 g of water, 35 g of apple juice, and 10 g of banana.

All ingredients were homogenized using a household blender (HENSKE, Vilnius, Lithuania; made in China) for 30 s at maximum speed followed by 20 s at minimum speed. The resulting smoothies were transferred into glass containers and pasteurized in a water bath at 80 °C for 20 min. After pasteurization and prior to probiotic inoculation, the microbiological status of the smoothie matrix was assessed by surface plating on Plate Count Agar (Liofilchem, Roseto degli Abruzzi, Italy), MRS and acidified Potato Dextrose Agar (Liofilchem, Roseto degli Abruzzi, Italy), and MRS agar supplemented with 0.05% L-cysteine to determine total aerobic mesophilic bacteria, yeasts and molds, and lactic acid bacteria, respectively. No detectable microbial growth was observed under applied conditions. After cooling to room temperature, the pasteurized smoothie matrices were inoculated with the respective bacterial suspensions prepared as described in Section 2.2, at a final concentration of 0.5% (*v*/*v*), followed by thorough mixing.

Probiotic viability and technological properties of the smoothies were evaluated over a storage period of 4 weeks at 4 °C. Prior to each analysis, samples were gently mixed to ensure homogeneity. Four batches of each smoothie were prepared independently.

2.4. Reducing Sugar Content

Reducing sugar (RS) content was measured by the 3,5-dinitrosalicylic acid (DNS) method based on Miller [21], with minor modifications. For analysis, 2 g of smoothie sample were mixed with 100 mL of distilled water and stirred for 10 min. The obtained dispersion was then centrifuged at 1200× *g* for 15 min at room temperature using a Microcen 23 centrifuge (Ortoalresa, Madrid, Spain). Next, 1 mL of the supernatant was transferred to a test tube and mixed with 1 mL of DNS reagent. The reaction mixture was heated in a water bath at 95 °C for 5 min, then cooled and brought to volume by adding 6 mL of distilled water. Absorbance was measured at 540 nm using a Genesys 10 spectrophotometer (Thermo Electron LED GmbH, Langenselbold, Germany). Quantification of reducing sugars was performed using a calibration curve prepared from glucose standard solutions (1 mg/mL), covering a concentration range of 0–1 mg/mL.

2.5. Probiotic Bacteria Viability Evaluation

The viability of *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium animalis* DSM 20105 in smoothies was monitored at weekly intervals over 4 weeks of storage. For microbiological analysis, 10 g of smoothie sample were aseptically mixed with 90 mL of sterile 0.9% NaCl solution and homogenized. Decimal serial dilutions were prepared, and

viable counts were determined by the plate count method. Enumeration was performed on MRS agar supplemented with 0.05% L-cysteine, followed by anaerobic incubation at 37 °C for 72 h. The results were expressed as $\log_{10}$ CFU/g.

2.6. Probiotic Bacteria Viability Under Simulated Gastrointestinal Conditions

The survival of *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium animalis* DSM 20105 in smoothies under simulated gastrointestinal conditions was evaluated at the end of storage using the INFOGEST protocol [22] with minor modifications. Electrolyte stock solutions for the oral, gastric, and intestinal phases were prepared at 1.25× concentration and diluted to 1× during the digestion assay. The simulated salivary fluid (SSF) contained (mmol/L): 15.1 KCl, 3.7 KH_2PO_4, 13.6 $NaHCO_3$, 0.15 $MgCl_2{\cdot}6H_2O$, 0.06 $(NH_4)_2CO_3$, and 1.1 HCl. The simulated gastric fluid (SGF) consisted of (mmol/L): 6.9 KCl, 0.9 KH_2PO_4, 25 $NaHCO_3$, 47.2 NaCl, 0.12 $MgCl_2{\cdot}6H_2O$, 0.5 $(NH_4)_2CO_3$, and 15.6 HCl. The simulated intestinal fluid (SIF) was composed of (mmol/L): 6.8 KCl, 0.8 KH_2PO_4, 85 $NaHCO_3$, 38.4 NaCl, 0.33 $MgCl_2{\cdot}6H_2O$, and 8.4 HCl. A separate 0.3 M $CaCl_2$ solution was prepared and added during digestion in order to avoid precipitation.

For the oral step, 5 g of smoothie were mixed with SSF at a 1:1 ratio, without amylase, and kept in a shaking water bath at 37 °C (100 rpm) until the sample was completely dispersed. Gastric digestion was then initiated by adding 7.5 mL SGF, 1.6 mL pepsin solution (25,000 AU/mL), 5 µL of 0.3 M $CaCl_2$, 0.05 mL of 6 M HCl, and 0.845 mL sterile distilled water. The pH was adjusted to 2.5 ± 0.1, and the mixture was incubated for 2 h at 37 °C with shaking at 100 rpm. To simulate the intestinal phase, the gastric digest was combined with 11 mL SIF, 5 mL pancreatin solution (800 AU/mL), 2.5 mL bile extract (9% ox bile), 40 µL of 0.3 M $CaCl_2$, 0.15 mL of 1 N NaOH, and 1.31 mL sterile distilled water, followed by incubation for another 2 h under the same temperature and shaking conditions.

Samples were taken after each digestion phase for microbial enumeration. Viable counts were determined by the standard plate count method after anaerobic incubation at 37 °C for 72 h, and the results were reported as $\log_{10}$ CFU/g.

2.7. Physicochemical and Colour Characteristic of Smoothies

The physicochemical properties of the smoothies were assessed by measuring pH and total titratable acidity (TTA). For both determinations, 10 g of smoothie sample were mixed thoroughly with 90 mL of distilled water. The pH value was then recorded using a pH meter (WinLab® Excellent Line, Clausthal-Zellerfeld, Germany). Titratable acidity was determined by titration with 1 N NaOH to an endpoint of pH 8.5 and expressed as the volume (mL) of alkali consumed.

Colour was analysed with a colorimeter (Konica Minolta, Tokyo, Japan) using the CIE Lab system. In this system, L describes lightness on a scale from 0 (black) to 100 (white), a indicates the green–red axis, and b* represents the blue–yellow axis. For measurement, smoothie samples were placed on a plate, and colour readings were taken at three randomly selected points on the sample surface.

2.8. Sensory Analysis

Sensory analysis of the smoothies was carried out in accordance with ISO 6658 [23] using a panel of 19 assessors recruited from students and staff of Kaunas University of Technology (Kaunas, Lithuania) (7 men and 12 women, 22–45 years of age). Before testing, the assessors attended a familiarization session in which the evaluated attributes, score definitions, and rating scales applied in the study were explained. Participation was limited to individuals who agreed to take part and who did not have temporary conditions that could interfere with sensory perception at the time of evaluation. Sensory evaluation was performed only on freshly prepared smoothies (day 0) to assess their initial sensory profile

and overall acceptability, while physicochemical properties changed only slightly during storage and probiotic viability varied at later storage stages. Each smoothie portion (50 mL) was labelled with a random three-digit code and served individually in monadic order. Water was provided to the assessors for palate cleansing between samples. All evaluations were performed at room temperature.

The assessed attributes included aroma, colour, cranberry taste, sourness, sweetness, and thickness, which were scored using a 7-point intensity scale, where 1 indicated the lowest and 7 the highest intensity. In addition, the samples were rated for sensory acceptability using a 7-point hedonic scale ranging from 1 ("dislike extremely") to 7 ("like extremely").

2.9. Statistical Analysis

Analyses were performed in quadruplicate ($n = 4$). Statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test at a significance level of $p \leq 0.05$. Data processing was carried out using Statgraphics Centurion 19 software (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results and Discussion

3.1. Quality Parameters of Smoothies

Cranberry pomace (CP) was used as a fiber-enriching ingredient in smoothie formulations. Smoothies were produced with 5% enzymatically hydrolyzed CP treated with either Celluclast® 1.5 L (0.02 mL/g) or Viscozyme® L (0.04 mL/g). The enzyme preparations and their concentrations were selected based on previous characterization results [9], in which these two hydrolysates represented compositionally distinct cranberry pomace ingredients. Celluclast® 1.5 L-treated pomace was characterized by a higher soluble dietary fiber (SDF) content (11.90 g/100 g dry matter) and 60.52 g/100 g dry matter of insoluble dietary fiber (IDF), whereas Viscozyme® L-treated pomace contained a higher amount of oligosaccharides (7.16 g/100 g dry matter), together with 56.46 g/100 g dry matter of IDF and 2.64 g/100 g dry matter of SDF. Thus, the selected hydrolysates differed in their relative proportions of SDF and low-molecular-weight carbohydrates, which was considered relevant for their behavior as smoothie ingredients and for their potential influence on product quality and probiotic performance. The incorporation of 5% Celluclast® or Viscozyme® hydrolysed CP increased the dietary fiber content of the smoothies by 3.62 and 3.26 g/100 g, respectively, including oligosaccharides. According to Regulation (EC) No 1924/2006, foods containing at least 3 g of fiber per 100 g may be labelled as a source of fiber; therefore, these smoothie formulations can be considered a source of fiber.

The incorporation of CP hydrolyzed with Celluclast® 1.5 L or Viscozyme® L significantly affected the acidity of the smoothies (Table 1). The lowest pH value and the highest TTA were observed in SCPV. The highest pH value and the lowest TTA were found in the control sample without pomace (SC). The lower pH and higher TTA observed in smoothies containing Viscozyme® L-treated pomace may reflect a greater release of acidic soluble compounds during enzymatic hydrolysis [24]. This may be particularly relevant for Viscozyme® L, which contains pectinolytic activities [25], as degradation of pectic polysaccharides can promote the release of acidic pectic components [24]. Since the whole hydrolysate was incorporated into the smoothies, differences in hydrolysate composition could directly influence product acidity.

Acidity is an important quality parameter of smoothies, influencing not only sensory acceptability but also microbiological safety. Previous studies have reported pH values ranging from 3.2 to 4.1 for various berry- and fruit-based smoothies. The pH of smoothies

largely depends on formulation, particularly the type and proportion of berries and fruits used, which differ in their natural organic acid composition [26].

Table 1. Color characteristics, pH, and TTA of enzymatically hydrolyzed cranberry pomace and fresh smoothies prepared with the corresponding pomace hydrolysates.

Parameters	Pomace		Smoothies		
	Celluclast® 1.5 L	Viscozyme® L	SC	SCPC	SCPV
L*	36.38 ± 0.29 ^{A}	41.04 ± 0.35 ^{B}	68.81 ± 1.07 ^{c}	33.34 ± 0.33 ^{a}	35.57 ± 0.58 ^{b}
a*	24.70 ± 0.19 ^{B}	22.13 ± 0.19 ^{A}	1.91 ± 0.25 ^{a}	17.06 ± 0.16 ^{c}	16.48 ± 0.08 ^{b}
b*	7.38 ± 0.07 ^{A}	8.84 ± 0.10 ^{B}	15.52 ± 1.19 ^{c}	5.61 ± 0.09 ^{a}	6.29 ± 0.06 ^{b}
pH	-	-	3.73 ± 0.01 ^{c}	3.22 ± 0.01 ^{b}	3.05 ± 0.02 ^{a}
TTA, mL 0.1 N NaOH/10 g	-	-	3.5 ± 0.1 ^{a}	8.4 ± 0.1 ^{b}	10.0 ± 0.1 ^{c}

Data values are presented as mean ± SD (n = 4). Different lowercase letters (a–c) within a row denote significant differences among fresh smoothies, while different uppercase letters (A–B) denote significant differences among enzymatically hydrolyzed pomace samples ($p < 0.05$). SC, control smoothie; SCPV, smoothie with cranberry pomace hydrolyzed with Viscozyme® L; SCPC, smoothie with cranberry pomace hydrolyzed with Celluclast ® 1.5 L.

Color parameters of smoothies (Table 1) showed that pomace addition decreased product lightness (L*) and yellowness (b*) while increasing redness (a*). The pomace color depended on the enzyme used for hydrolysis, which was reflected in the color differences between SCPV and SCPC. Berries are rich in various polyphenols and flavonoids, such as anthocyanins, which are responsible for their color and antioxidant properties. Anthocyanins, predominantly located in the berry skins, confer the characteristic bright red color to cranberry berries, whereas flavonoids, which are secondary yellowish compounds distributed throughout the entire berry, do not have a significant impact on its color [27]. A substantial proportion of these polyphenolic and flavonoid compounds remains associated with the solid fraction after juice extraction, resulting in their retention in berry pomace. These color changes may be attributed to the release of color-related compounds during enzymatic hydrolysis, as such compounds are often bound within the plant cell wall matrix together with other components and are therefore difficult to extract [28].

Sensory evaluation of fresh smoothies revealed that the products enriched with CP were perceived as having higher sourness and thickness, but lower sweetness and aroma intensity compared to the control (Figure 1). SCPV was characterized by the least acceptable sourness and sweetness and by the most intense cranberry taste. The control sample showed the most acceptable aroma and sweetness. SCPC was rated as having the highest thickness, the most acceptable color, and cranberry taste. The sample without CP was evaluated as the sweetest. Overall, all smoothies were liked by the assessors more than moderately, and no statistically significant differences in overall acceptability were found among the samples. Similar results have been reported in a previous study in which berry pomace was used for smoothie formulation. In that study, products formulated both with and without pomace received acceptable sensory evaluation scores [16].

3.2. Viability of Probiotic Bacteria During Storage

The effect of enzymatically hydrolyzed CP on the viability of probiotic bacteria in smoothies during storage is shown in Table 2. To ensure consumer convenience, bacterial viability and quality parameters were assessed at 7-day intervals. Results revealed that viability was influenced by both the addition of pomace and by the probiotic strain used.

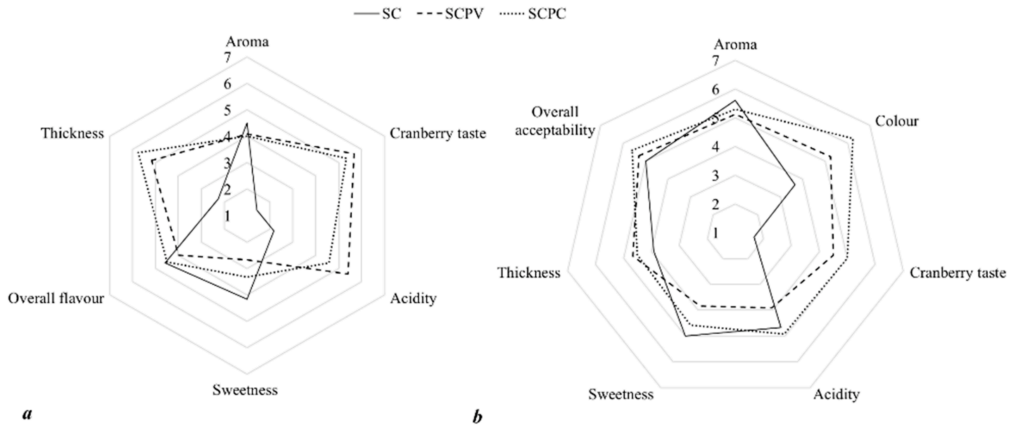

Figure 1. Sensory analysis of smoothies: (**a**) intensity and (**b**) acceptability. SC, control smoothie; SCPV, smoothie with cranberry pomace hydrolyzed with Viscozyme® L; SCPC, smoothie with cranberry pomace hydrolyzed with Celluclast ® 1.5 L.

Table 2. Storage stability of *B. animalis* DSM 20105 and *L. acidophilus* DSM 20079 in probiotic smoothies.

Storage Time, d	*B. animalis* DSM 20105, $\log_{10}$ CFU/g			*L. acidophilus* DSM 20079, $\log_{10}$ CFU/g		
	SC	SCPC	SCPV	SC	SCPC	SCPV
0	7.82 ± 0.06^{aA}	7.80 ± 0.23^{aA}	7.74 ± 0.20^{aC}	7.61 ± 0.04^{aC}	7.62 ± 0.06^{aB}	7.63 ± 0.09^{a}
7	7.85 ± 0.03^{dA}	7.82 ± 0.09^{dA}	6.93 ± 0.04^{cB}	5.76 ± 0.13^{bB}	2.79 ± 0.04^{aA}	-
14	7.77 ± 0.11^{cA}	7.81 ± 0.15^{cA}	6.91 ± 0.05^{bB}	3.66 ± 0.17^{aA}	-	-
21	7.79 ± 0.00^{bA}	7.80 ± 0.12^{bA}	6.87 ± 0.07^{aB}	-	-	-
28	7.62 ± 0.12^{bA}	7.59 ± 0.02^{bA}	5.76 ± 0.01^{aA}	-	-	-

Data values are expressed as means with standard deviation ($n = 4$). Different lowercase letters (a–d) means within a row with different superscripts are significantly different ($p < 0.05$). Uppercase letters (A–C) means within a column with different superscripts are significantly different ($p < 0.05$). SC, control smoothie; SCPV, smoothie with cranberry pomace hydrolysed with Viscozyme® L; SCPC, smoothie with cranberry pomace hydrolysed with Celluclast ® 1.5 L.

For *L. acidophilus* DSM 20079, a significant ($p < 0.05$) reduction in cell counts was observed during the first week of storage in all samples, with values dropping below the recommended level of 6 $\log_{10}$ CFU/g, which is generally considered the minimum viable count in probiotic products [29]. In SCPC, viability decreased to 2.79 $\log_{10}$ CFU/g after one week, whereas in SCPV no viable cells were detected. The highest viability after one week was observed in the control sample (SC) without pomace addition.

The decline in viability of *L. acidophilus* DSM 20079 was most likely related to the combined stress imposed by the CP, including the low pH and the presence of organic acids and other antimicrobial constituents naturally associated with CP [30]. *L. acidophilus* DSM 20079 is particularly sensitive to acidic conditions, as reflected in its reduced survival at pH 2 after 4 h of incubation [31]. Nualkaekul et al. [32] reported that lactic acid bacteria lost viability more rapidly in cranberry juice compared with orange, pineapple, or blackcurrant juice, attributing this effect to both the low pH and the presence of antimicrobial compounds such as phenolic acids (e.g., benzoic acid). Similarly, Mousavi et al. [33] found that *L. acidophilus* DSM 20079 lost viability in pomegranate juice (pH ≈ 3.3) within 2 weeks of storage, citing high acidity and low storage temperature (4 °C) as the main contributing factors. The poorer survival of this strain in pomace-containing formulations compared with the control may therefore indicate that the additional acidity and bioactive constituents introduced with cranberry pomace outweighed any possible protective effect of the fiber-rich ingredient.

These results also suggest that the suitability of probiotic strains for cranberry pomace-enriched smoothie is strongly strain-dependent. In the present study, *L. acidophilus* DSM

20079 appeared poorly adapted to this acidic fruit matrix, regardless of formulation. To incorporate this strain into such products, increasing the initial cell count or reducing the storage period could be considered. While various immobilization techniques are often employed to protect probiotic cells, encapsulation may negatively affect sensory attributes and consumer acceptance of the product.

The viability of *B. animalis* DSM 20105 in smoothies was higher than that of *L. acidophilus* DSM 20079; however, a gradual decline during storage was still observed. The greatest reduction occurred in SCPV, where viability after 4 weeks dropped below 6 $\log_{10}$ CFU/g (a decrease of 1.74 $\log_{10}$ CFU/g). Nevertheless, after 3 weeks of storage, the viable count in this product remained above 6 $\log_{10}$ CFU/g. Therefore, for SCPV, either the shelf life should be shortened or a higher initial inoculum should be applied to maintain probiotic levels after 4 weeks of storage. In SC and SCPC, viability after 4 weeks remained above 6 $\log_{10}$ CFU/g.

The higher survival of *B. animalis* DSM 20105 may be partly explained by the fact that the water-soluble fraction of cranberry pomace hydrolyzed with Celluclast® 1.5 L exhibited a stronger growth-promoting effect for this strain compared with the fraction hydrolyzed with Viscozyme® L [9]. This may indicate that the composition of the hydrolyzed pomace influenced probiotic maintenance in the smoothie matrix. In particular, the better survival observed in SCPC than in SCPV may be related to differences in the soluble fraction generated by enzymatic treatment, whereas the lower viability in SCPV may additionally reflect the less favorable physicochemical conditions of this formulation, including its lower pH and higher titratable acidity. Sheehan et al. [34] also reported a significant reduction in the viability of *Bifidobacterium animalis* subsp. *lactis* Bb-12 in cranberry juice within one week of storage, attributing this decline to the acidity of the juice and the presence of antimicrobial compounds. DF may contribute to probiotic survival through several mechanisms, including matrix-related protection, increased viscosity of the surrounding environment, provision of attachment sites for bacterial cells, and prebiotic effects associated with selectively utilizable carbohydrates. In particular, soluble dietary fiber may create a more protective microenvironment and support cell adhesion or biofilm formation within the fiber matrix, which could contribute to improved probiotic maintenance [35,36]. The poorer survival observed in the SCPV formulation may also be associated with the higher phenolic content of Viscozyme® L-treated cranberry pomace. According to previous characterization [9], the total phenolic content of Viscozyme® L-treated pomace was 7.81 $\pm$ 0.36 mg GAE/g, whereas Celluclast® 1.5 L-treated pomace contained 7.06 $\pm$ 0.28 mg GAE/g. Since phenolic compounds may exert concentration-dependent antimicrobial effects [37], the higher phenolic content of the Viscozyme®-treated pomace could have contributed to the lower viability observed in this formulation.

3.3. Changes in Smoothie Quality Parameters During Storage

The quality parameters of probiotic and dietary fiber-enriched smoothies, such as acidity and sugar content, may change during storage due to bacterial metabolic activity. Such changes are usually associated with a decrease in sugars (primarily glucose) and an increase in acidity. The pH, TTA, and reducing sugar content of the smoothies during storage are shown in Table 3.

Acidity of smoothies was influenced only by the addition of enzymatically hydrolyzed CP. These parameters remained stable throughout storage. Since the viability of *L. acidophilus* DSM 20079 decreased below 6 $\log_{10}$ CFU/g after 7 days, the quality parameters of products containing this strain were not evaluated at later storage points.

The reducing sugar (RS) content of smoothies depended on the enzyme preparation used for pomace hydrolysis. At day 0, the highest RS content was determined in SCPV,

while the lowest was found in SC. During storage, RS content increased slightly. Among the products enriched with *B. animalis* DSM 20105, the most pronounced RS changes were observed in SCPC.

Table 3. pH, TTA, and reducing sugar content of probiotic smoothies during storage.

Storage Time, Days	*B. animalis* DSM 20105			*L. acidophilus* DSM 20079		
	SC	**SCPC**	**SCPV**	**SC**	**SCPC**	**SCPV**
pH						
0	3.73 ± 0.01^{cA}	3.22 ± 0.01^{bA}	3.05 ± 0.02^{aA}	3.73 ± 0.01^{cA}	3.22 ± 0.01^{bA}	3.05 ± 0.02^{aA}
7	3.70 ± 0.01^{cA}	3.22 ± 0.01^{bA}	3.09 ± 0.02^{aA}	3.70 ± 0.01^{cA}	3.22 ± 0.01^{bA}	3.09 ± 0.01^{aA}
14	3.70 ± 0.02^{cA}	3.24 ± 0.02^{bA}	3.08 ± 0.02^{aA}	-	-	-
21	3.73 ± 0.01^{cA}	3.24 ± 0.02^{bA}	3.06 ± 0.01^{aA}	-	-	-
28	3.70 ± 0.01^{cA}	3.21 ± 0.01^{bA}	3.04 ± 0.01^{aA}	-	-	-
TTA, mL 0.1 N NaOH/10 g						
0	3.5 ± 0.1^{aA}	8.4 ± 0.1^{bA}	10.0 ± 0.1^{cA}	3.5 ± 0.1^{aA}	8.4 ± 0.1^{bA}	10.0 ± 0.1^{cA}
7	3.5 ± 0.1^{aA}	8.4 ± 0.1^{bA}	10.0 ± 0.0^{cA}	3.5 ± 0.2^{aA}	8.4 ± 0.1^{bA}	10.1 ± 0.1^{cA}
14	3.5 ± 0.1^{aA}	8.4 ± 0.2^{bA}	10.0 ± 0.1^{cA}	-	-	-
21	3.5 ± 0.1^{aA}	8.4 ± 0.2^{bA}	10.0 ± 0.1^{cA}	-	-	-
28	3.5 ± 0.1^{aA}	8.4 ± 0.1^{bA}	10.0 ± 0.1^{cA}	-	-	-
RS, g/100 g						
0	3.46 ± 0.09^{aA}	4.13 ± 0.06^{bA}	4.36 ± 0.05^{cA}	3.46 ± 0.09^{aA}	4.13 ± 0.06^{bA}	4.36 ± 0.05^{cA}
7	3.51 ± 0.07^{aAB}	4.98 ± 0.06^{cB}	4.62 ± 0.12^{bB}	3.48 ± 0.06^{aA}	4.54 ± 0.03^{bB}	4.59 ± 0.04^{bB}
14	3.63 ± 0.03^{aAB}	5.13 ± 0.17^{cB}	4.78 ± 0.07^{bB}	-	-	-
21	3.67 ± 0.12^{aB}	5.16 ± 0.17^{bB}	5.04 ± 0.14^{bC}	-	-	-
28	3.47 ± 0.03^{aA}	4.95 ± 0.10^{bB}	5.36 ± 0.03^{cD}	-	-	-

Data values are expressed as means with standard deviation ($n = 4$). Different lowercase letters (a–c) means within a row with different superscripts are significantly different ($p < 0.05$). Uppercase letters (A–D) means within a column with different superscripts are significantly different ($p < 0.05$). SC, control smoothie; SCPV, smoothie with cranberry pomace hydrolyzed with Viscozyme® L; SCPC, smoothie with cranberry pomace hydrolyzed with Celluclast ® 1.5 L.

Throughout storage, pH and TTA remained stable, suggesting that no pronounced post-processing acidification occurred and that the added probiotic bacteria did not substantially utilize the available sugars. Similar findings have been reported in other studies, where smoothies and probiotic-enriched beverages exhibited only minor changes in pH, TTA, and sugar content, indicating limited or no utilization of sugars by the added bacteria [26,38]. Nevertheless, slight increases in reducing sugars during storage have also been observed in fruit-based and probiotic beverages, suggesting that carbohydrate transformations may continue even when acidification is limited. In acidic fruit matrices, non-reducing sugars such as sucrose may gradually hydrolyze into glucose and fructose, thereby increasing the reducing sugar fraction, and this process may be further intensified during storage as a result of prolonged storage time and temperature effects [39,40].

The slight increase in RS content in the present study could be associated with bacterial cell lysis during storage and the subsequent release of intracellular carbohydrate-hydrolyzing enzymes into the smoothie matrix. Bifidobacteria are characterized by a broad range of carbohydrate-active enzymes, including glycoside hydrolases such as galactosidases and glucosidases, which are involved in the degradation of carbohydrates [41,42]. As bacterial viability declined during storage, loss of cell integrity may have promoted the release of these intracellular enzymes into the medium following cell lysis [43]. Once released, such enzymes may have contributed to further hydrolysis of available carbohydrate substrates and, consequently, to the slight increase in RS.

3.4. Survival of Bifidobacterium animalis DSM 20105 Under Simulated Gastrointestinal Conditions

During in vitro digestion, the viability of *B. animalis* DSM 20105 decreased in all samples (Table 4); however, the reduction was less pronounced in SCPC and SC (0.73 and 0.82 $\log_{10}$ CFU/g, respectively) compared with SCPV (1.47 $\log_{10}$ CFU/g). *B. animalis* DSM 20105 exhibited high resistance to low pH values [31], which explains its survival in gastric juice. Madureira et al. [44] similarly reported that *B. animalis* strains generally show little reduction in viability during in vitro digestion, although survival can depend on the specific strain and product matrix. In contrast, Soares et al. [45] found that *B. animalis* added to orange juice was more sensitive to acidic conditions, resulting in a significant loss of viability. In addition to pH, digestive enzymes play an important role: pepsin exerts a stronger effect on bacterial viability than pancreatin. Pepsin cleaves peptide bonds between amino acids, thereby disrupting bacterial cell membranes, whereas pancreatin primarily acts on smaller protein molecules. Furthermore, bile salts influence bacterial survival in the intestinal phase, as they can damage cell membranes [46,47].

Table 4. Survival of *B. animalis* DSM 20105 during in vitro digestion.

Simulated In Vitro Digestion Stage	*B. animalis* DSM 20105, $\log_{10}$ CFU/g		
	SC	SCPC	SCPV
Saline phase	7.64 ± 0.14^{cC}	7.60 ± 0.01^{bB}	5.79 ± 0.03^{aC}
Gastric phase	7.03 ± 0.02^{bB}	6.95 ± 0.07^{bA}	5.17 ± 0.05^{aB}
Intestine phase	6.82 ± 0.09^{bA}	6.87 ± 0.04^{bA}	4.32 ± 0.03^{aA}

Data values are expressed as means with standard deviation ($n = 4$). Different lowercase letters (a–c) means within a row with different superscripts are significantly different ($p < 0.05$). Uppercase letters (A–C) means within a column with different superscripts are significantly different ($p < 0.05$). SC, control smoothie; SCPV, smoothie with cranberry pomace hydrolysed with Viscozyme® L; SCPC, smoothie with cranberry pomace hydrolysed with Celluclast ® 1.5 L.

The differences observed among the tested formulations suggest that probiotic survival during digestion was influenced not only by strain tolerance, but also by digestion-induced changes in the smoothie matrix. In addition to the direct effects of low pH, digestive enzymes, and bile salts, gastrointestinal digestion may alter the plant matrix and promote the release of bioaccessible compounds from the incorporated pomace. Previous studies with fruit- and vegetable-based by-product matrices have shown that digestion can increase the release of antioxidant compounds, particularly polyphenols, from complex plant structures [48]. At the same time, soluble and insoluble polysaccharides may increase matrix viscosity and slow the release and solubilization of compounds during digestion, which could reduce the direct exposure of probiotic cells to harsh gastrointestinal conditions [37,48]. In this context, the lower viability loss observed in SCPC may indicate a more favorable matrix effect during digestion. In contrast, the greater reduction observed in SCPV may be related to the release of a less favorable bioaccessible fraction during digestion, since Viscozyme® L-treated pomace previously showed a higher total phenolic content than Celluclast® 1.5 L-treated pomace.

Among the tested formulations, the smoothie containing Celluclast® 1.5 L-treated pomace appeared the most promising, as it combined favorable sensory properties with better maintenance of *B. animalis* DSM 20105 viability during storage and simulated gastrointestinal digestion. In contrast, the formulation containing Viscozyme® L-treated pomace appeared less promising because of its lower pH, higher TTA, and less favorable probiotic survival.

4. Conclusions

Enzymatically hydrolyzed cranberry pomace influenced the quality characteristics and probiotic viability of smoothies. Among the tested formulations, smoothies prepared with Viscozyme® L-hydrolyzed pomace showed the lowest pH and the highest total titratable acidity, whereas acidity remained stable during 28 days of storage at 4 °C. The smoothie containing Celluclast® 1.5 L-hydrolyzed pomace showed the most favorable sensory profile, being characterized by the highest thickness, the most acceptable color, and the most pronounced cranberry taste. Probiotic viability differed between the tested strains: *B. animalis* DSM 20105 maintained viable counts above 6 $\log_{10}$ CFU/g for 28 days in the control smoothie and in the smoothie containing Celluclast® 1.5 L-hydrolyzed pomace, whereas *L. acidophilus* DSM 20079 declined below this level during the first week of storage. During simulated gastrointestinal digestion, viable counts of *B. animalis* DSM 20105 remained above 6 $\log_{10}$ CFU/g in the control smoothie and in the smoothie containing Celluclast® 1.5 L-hydrolyzed pomace. Overall, the combination of *B. animalis* DSM 20105 and Celluclast® 1.5 L-hydrolyzed cranberry pomace showed the most favorable overall performance among the tested samples. These results show that cranberry pomace preparations obtained using different enzymatic treatments were associated with different effects on smoothie quality characteristics and probiotic viability.

The obtained results highlight the potential of enzymatically hydrolyzed cranberry pomace for the development of probiotic smoothies. Further studies involving a broader range of probiotic strains and smoothie compositions would allow for a more comprehensive evaluation of the relationships between pomace modification, product quality, and probiotic survival during storage and simulated gastrointestinal digestion, and would further support the applicability of these products in a broader industrial context.

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Abbreviations

The following abbreviations are used in this manuscript:

CP	cranberry pomace
RS	reducing sugars
SC	control smoothie without cranberry pomace
SCPV	smoothie with cranberry pomace hydrolyzed with Viscozyme® L.
SCPC	smoothie with cranberry pomace hydrolyzed with Celluclast ® 1.5 L.
TTA	total titratable acidity

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