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Enzymatic Modification of Apple Pomace and Its Application in Conjunction with Probiotics for Jelly Candy Production

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Abstract: This study aimed to evaluate the applicational possibilities of enzymatically modified apple pomace (AP) in conjunction with probiotics as value-added ingredients for the production of jelly candies. AP was enzymatically modified with Pectinex® Ultra Tropical, Viscozyme® L, and Celluclast® 1.5 L (Novozyme A/S, Bagsværd, Denmark), and the soluble and insoluble dietary fibre content was determined using the Megazyme kit (Megazyme International Ireland Ltd., Wicklow, Ireland), reducing sugar content using the 3,5-dinitrosalicylic acid assay. The technological properties of the modified AP, such as its swelling capacity, water-retention capacity, oil-retention capacity, bulk density, and static and thermal emulsion stability, were evaluated. Enzymatically modified AP hydrolysed with Celluclast® 1.5 L was used for the production of jelly candies supplemented with *Bifidobacterium animalis* DSM 20105. The survival of probiotics in the jelly candies during in vitro digestion, the viability of probiotics during candy storage, and candy quality characteristics were analysed. Enzymatically modified AP had different carbohydrate compositions and technological properties, depending on the enzyme preparation used. Although the viability of probiotics in the jelly candies decreased during storage, a significantly higher viability of *B. animalis* was determined in jelly candies supplemented with hydrolysed AP compared with control candies made without AP after digestion in the saline, gastric, and intestine phases. This study shows that Celluclast® 1.5 L can be used for increasing the soluble dietary fibre in AP (18.4%), which can be further applied, in conjunction with *B. animalis*, for added-value jelly candy production.

Keywords: dietary fibre; enzymatic hydrolysis; probiotic viability; added-value product

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1. Introduction

In 2020, the European Commission launched the Farm to Fork strategy, which aims to create a fair, reliable, and environmentally friendly food system. This strategy aims to reduce the climate impact of the European Union food system. In order to create sustainable production and a more efficient use of by-products of the food industry, attention has been paid to the possibility of applying berry and fruit pomace as a source of dietary fibre (DF) in various food products [1–3]. According to the recommendation of the EFSA, an adult should consume 25 g of DF per day [4]; therefore, the enrichment of food products with fibre from berries or fruit pomaces may solve both problems: sustainability and health issues by foods with a higher nutritional value.

Apples are one of the most popular fruits in the world, which, when used in juice production, generate large amounts of pomace, representing approximately 25–35% of the total apple mass [5,6]. The pomace obtained from apple could be a value-added ingredient,

as it is a source of antioxidant phytochemicals, polyphenols, minerals, vitamins, and DF [3,7–10]. The chemical composition of pomace, including the DF content and the ratio between the soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) (recommended to be 1:2 [11]), are important for nutrition and depend on the source and processing conditions [12,13]. An SDF intake of a minimum of 6 g/day is associated with several health benefits, like a reduction in low-density lipoprotein cholesterol in serum, glycemic control, and a reduction in the risk of coronary heart disease [14,15]. In order to use pomace in the production of food products, not only is their composition important but also the technological properties that determine the quality of the final product. Modifications of the pomace with enzymes could not only change the SDF/IDF ratio by increasing the amount of SDF content but could also improve the technological properties [12].

Despite having good nutritional value, apple pomace is primarily used as animal feed, resulting in its loss within the food chain. Several studies have valorised apple pomace (AP) through enzymatic hydrolysis, focusing primarily on the yield of bioactive compounds and fermentable sugars. However, there is less information available regarding the modification of whole pomace and its application in novel food products. An innovative enzymatic modification of AP has the potential to transform it into valuable resources while also addressing pressing environmental concerns [16,17]. Mostly, AP as a functional ingredient, is used in bakery products such as bread, cookies, biscuits, muffins. AP not only increases the dietary fibre content but also provides acceptable sensory properties [18–21]. On the other hand, there is limited information on the application of AP in other products that could be consumed daily and that appeal to various consumer groups (e.g., jelly candy).

Foods with a higher nutritional value can also be supplemented with probiotics. The health benefits of probiotics have been known for a long time, and the popularity of products supplemented with these beneficial bacteria is increasing [22]. The most common and industrially applicable microorganisms applied for food production as probiotic microorganisms are lactic acid bacteria of the *Lactobacillus* genus, whereas less commonly used probiotics belong to the *Bifidobacteria* genus [23]. For the development of novel probiotic products, one of the essential factors is the selection of proper cultures. Each probiotic has to be safe, survive in the gastrointestinal track environment, possess beneficial properties, and be effectively used [22].

The literature contains data on products supplemented with probiotics or pomace as a source of DF, but, less often, various products are supplemented with both probiotics and fruit or berry pomace, particularly enzymatically hydrolysed pomace, as a DF source. Therefore, it remains relevant to evaluate the possibilities of the complex use of pomace and probiotics for the development of new products with a higher nutritional value. Products such as various candies, gummies, or chewing gum are convenient food matrices that can be supplemented with dietary fibre and probiotics [24]. The global confectionery industry encompasses a diverse array of products and was valued at approximately USD 298.23 billion in 2021. This market is anticipated to grow at a compound annual growth rate (CAGR) of 4.3% during the period from 2022 to 2028 [25]. From a nutritional perspective, jelly candies are classified as unhealthy sugary treats with a low antioxidant content. As a result, creating healthier alternatives poses significant challenges. Current approaches focus on replacing sugar with sweeteners and/or incorporating dietary fibre into their formulations [26,27]. However, limited attention has been given to the use of enzymatically hydrolysed pomace as a functional ingredient in confectionery products. Its application in food product development remains underexplored, particularly in the context of functional confectionery, presenting opportunities to create innovative products that combine health benefits with sustainability. While several studies have focused on valorising AP through enzymatic hydrolysis, primarily targeting bioactive compounds

and fermentable sugars [16,17], the combination of enzymatically hydrolysed AP with probiotics, particularly in functional confectionery, has been insufficiently investigated, emphasizing the novelty of this approach. Therefore, the objective of this study was to analyse the dried pomace powder from apple juice extraction (AP) as sources of DF and its modification possibilities using commercial enzymes (Viscozyme® L, Pectinex® Ultra Tropical, and Celluclast® 1.5 L). This study was designed to investigate the composition of carbohydrates and technological properties (swelling capacity, water-retention capacity, oil-retention capacity, bulk density, and static and thermal emulsion stability) of the enzymatically modified AP. Moreover, enzymatically modified AP was applied in the jelly candy recipe as a value-added ingredient supplemented with the probiotic *Bifidobacterium animalis* DSM 20105 strain. The integration of enzymatically hydrolysed pomace into confectionery formulations not only addresses the demand for foods with a higher nutritional value but also represents an innovative approach to valorising fruit by-products in a sustainable manner. This study provides useful insights into the similarities and differences between the composition of carbohydrates and the technological properties of AP modified with different commercial enzymatic preparations and will aid in the selection of suitable dietary fibre-rich AP products as functional ingredients for further applications in foods. Moreover, this study provides information on *B. animalis* survival in jelly candy during in vitro digestion, the viability of probiotics during storage, and the quality characteristics of candy made with modified AP products.

2. Materials and Methods

2.1. Apple Pomace

Powdered apple pomace was purchased from "UOGUSULTYS" (Giedriai, Lithuania). The moisture content was 8.46%, and the particle size was <1 mm. The chemical composition was as follows: crude protein, 3.59 ± 0.05%; lipid, 1.38 ± 0.15%; ash, 2.43 ± 0.08%; dietary fibre, 35.66 ± 1.25%; reducing sugars, 46.59 ± 0.93%, on a dry-weight basis.

2.2. Enzymatic Hydrolysis

AP was mixed with distilled water at a ratio of 1:10, and enzymatic hydrolysis was carried out using the commercial enzymes Viscozyme® L (Viscozyme, 100 FBG/g), composed of β-glucanases, pectinases, hemicellulases, and xylanases (enzyme/substrate (pomace) (E/S) ratio of 0.02 mL/g) [28]; Pectinex® Ultra Tropical (Pectinex, 5000 PECTU/g), composed of pectinases, cellulases, hemicellulases, and β-glucanases (E/S ratio of 0.02 mL/g) [29]; Celluclast® 1.5 L (Celluclast, 700 EGU/g), composed of cellulases (E/S ratio of 0.04 mL/g) (Novozyme, Bagsværd, Denmark). The concentrations of enzymes were chosen on the manufacturer's recommendations and literature data [30,31]. The hydrolysis was performed at 50 °C (according to the manufacturer's recommendations) for 1 and 5 h with shaking (200 rpm). After treatment, samples were heated in a 95 °C water bath for 20 min and were cooled at room temperature. Enzymatically hydrolysed samples were freeze-dried (Harvest Right, North Salt Lake, UT, USA) and kept in a dark place at room temperature until analysis.

2.3. Evaluation of Dietary Fibere and Reducing Sugars

The total dietary fibre (TDF), SDF, and IDF were determined based on the American Association of Cereal Chemists (AACC) method 32-07.01 and the AOAC method 991.43 using Megazyme's Total Dietary Fiber assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) and according to the manufacturer's instructions.

The reducing sugar (RS) content was determined using the 3,5-dinitrosalicylic acid (DNS) assay, according to Miller [32], with some modifications. The pomace (0.5 g) was

mixed with distilled water (100 mL) using a stirrer for 10 min. After that, the sample was centrifugated at 1200× *g* (Microcen 23, Ortoalresa, Madrid, Spain) for 15 min at room temperature. The obtained supernatant (1 mL) was mixed with a DNS reagent (1 mL) and was heated for 5 min at 95 °C. After that, the mixture was cooled and diluted using 6 mL of distilled water. The absorbance was measured at 540 nm using a spectrophotometer (Model Genesys 10, Thermo Electron LED GmbH, Langenselbold, Germany). The RS content was calculated from an equation of the standard curve. The standard curve was prepared using a glucose solution (1 mg/mL) and distilled water to obtain final glucose concentrations in the range of 0 to 1 mg/mL.

2.4. Technological Properties

2.4.1. Water-Retention Capacity (WRC), Water-Swelling Capacity (WSC), and Solubility Index (SI)

The WRC and WSC were determined using the method reported by Sheng et. al. [33], with some modifications. AP (0.2 g) was mixed with 6 mL of distilled water and was left for 18 h at room temperature. The volume of the pomace was recorded before and after hydration. After hydration, the sample was centrifugated at 1200× *g* (Microcen 23, Ortoalresa, Madrid, Spain) for 20 min, and hydrated residues were weighed before and after drying at 105 °C to a constant weight. The supernatant, after decantation, was dried at 105 °C to a constant weight. The SI was calculated by the following equation:

$$\text{SI (\%)} = (\text{M}/\text{M0}) \times 100 \quad (1)$$

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

The WSC was calculated by the following equation:

$$\text{WSC (mL/g)} = (\text{V1} - \text{V0})/\text{M0} \quad (2)$$

where V1 is the volume of the pomace after hydration (mL), V0 is the volume of the pomace before hydration (mL), and M0 is the weight of the dry pomace prior to hydration (g).

The WRC was calculated by the following equation:

$$\text{WRC (g/g)} = (\text{M1} - \text{M2})/\text{M2} \quad (3)$$

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

2.4.2. Oil-Retention Capacity (ORC)

The ORC was determined according to Sheng et al. [33], with some modifications. The pomace (0.2 g) was mixed with 2 g of sunflower oil and was left at room temperature for 1 h. After that, the mixture was centrifugated at 430× *g* (Microcen 23, Ortoalresa, Madrid, Spain) for 10 min, and the unabsorbed oil was carefully decanted, and the pellets were recovered and weighed. The ORC was calculated by the following equation:

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

where W1 is the weight of the pellets (g), and W0 is the weight of the dry sample (g).

2.4.3. Emulsion Stability

Emulsion was prepared in graduated tubes by mixing 0.16 g of the pomace with 8 mL of distilled water. Then, 8 g of sunflower oil was added. A rotor–stator homogenizer (IKA®

T-25 digital, Ultra-Turrax, Staufen, Germany) was used for emulsification for 5 min at 10,000 rpm. The emulsion was stored for 3 weeks at 4 °C. The static stability of the emulsion was recorded in terms of phase separation and is expressed by the following equation.

$$\text{Emulsion stability (\%)} = (VE/VT) \times 100 \quad (5)$$

where VE is the volume of emulsion (mL), and VT is the volume of the total sample (mL).

The thermal stability of emulsions was evaluated by heating the emulsions in a 80 °C water bath for 30 min. Then, the samples were cooled to room temperature and were stored for 2 weeks at 4 °C. The thermal stability of the emulsion was recorded and expressed as previously described.

2.4.4. Bulk Density

The bulk density was determined by weighing 1 g (m) of the dry sample into a graduated cylinder and by estimating the volume occupied (V). The bulk density was calculated according to the following equation:

$$\text{Bulk density (g/mL)} = m/V \quad (6)$$

where m is the weight of the dry sample (g), and V is the volume of the pomace (mL)

2.5. Preparation of Probiotic Jelly Candies

Bifidobacterium animalis DSM 20105 was cultured anaerobically in a DeMan, Rogosa, and Sharpe (MRS) broth (CM 0359, Oxoid Ltd., Basingstoke, Hampshire, UK) at 30 °C for 18 h. The cells were then collected by centrifugation (4000 g for 10 min) and washed twice with 9 g/L of a sterile NaCl solution. Jelly candies were prepared from an 85.77% enzymatically hydrolysed AP and water mixture (ratio 1:10; 7.79 g of AP and 77.98 g of water); 12.87% gelatine; a 1.29% sweetener mixture (99.8% erythritol and 0.2% steviol glycoside); and 0.07% citric acid (all ingredients were obtained from a local retailer). As a control, a jelly candy was prepared with water instead of AP. The AP and water mixture, after hydrolysis with Celluclast, was mixed with other components and was heated in a 95 °C water bath for 20 min until the gelatine was completely dissolved. After that, the AP and water mixture was cooled to 45 °C, and a 1% suspension of *Bifidobacterium animalis* DSM 20105 bacteria (10.2 log10 CFU/mL) was suspended. The mass was poured into containers and was cooled for 18 h at 4 °C. The probiotic jelly candies were stored in airtight plastic containers at 4 °C for 8 weeks.

2.6. Probiotic Bacteria Viability Evaluation

The viability of probiotic bacteria in jelly candies during storage was determined weekly during storage for 8 weeks. Briefly, 10 g of the sample was mixed with 90 mL of a diluent (0.9% NaCl solution), tenfold dilutions were performed, and bacteria counts were determined using the standard plate count technique. Bacteria were grown on an MRS agar supplemented with 0.05% L-cysteine under anaerobic conditions at 37 °C for 72 h. The count of probiotics is expressed as log10 CFU/g.

The viability of probiotic bacteria in jelly candies under in vitro digestion conditions was performed according to the INFOGEST protocol [34], with some modifications at the end of the storage. Jelly candies (5 g) were mixed with simulated salivary fluid (SSF) at a ratio of 1:1 without amylase in a 37 °C water bath with shaking at 100 rpm until the jelly candies dissolved. After that, the jelly candies/SSF mixture was mixed with 7.5 mL of simulated gastric juice, 1.6 mL of a pepsin solution (25,000 AU/mL), 5 µl of 0.3 M of $CaCl_2$, 0.05 mL of 6 M of HCl, and 0.845 mL of sterile distilled water (final pH 2.5 ± 0.1)

and incubated for 2 h in a 37 °C water bath with shaking at 100 rpm. After that, 11 mL of simulated intestinal fluid, 5 mL of a pancreatin solution (800 AU/mL), 2.5 mL of bile (9% ox bile), 40 µL of 0.3 M of $CaCl_2$, 0.15 mL of 1 N of NaOH, and 1.31 mL of sterile distilled water were added and incubated for 2 h with shaking (100 rpm) in a 37 °C water bath. After each stage, the samples were taken to assess their bacterial viability. The probiotic count was evaluated after 72 h of anaerobic incubation at 37 °C as log10 CFU/g.

2.7. Physicochemical Characteristics of Jelly Candies

The pH and total titratable acidity (TTA) of the jelly candies were determined from a 10 g sample homogenized with 90 mL of distilled water. The pH was determined directly using a pH meter (WinLab® Excellent Line, Clausthal-Zellerfeld, Germany). The TTA is expressed in millilitres of 1 mol/l of a NaOH solution used to obtain pH = 8.5.

The Texture Analyzer TA.XT plus (Stable Micro System, Godalming, UK) equipment with an SMS P/100 probe (100 mm aluminium cylinder) was used to determine the hardness (the force required to compress the sample, expressed as the maximum force of the first compression) of the candies. The candies with a 30 mm diameter and a thickness of 6 mm were compressed to 50% of their original height. The texture analysis was performed on the first day and after 56 days of storage.

The colour characteristics of the candies were evaluated using a colorimeter (Konica Minolta, Tokyo, Japan) on the first day and after 56 days of storage. The CIELAB colour space was used to evaluate the colour, in which the L* numerical value describes lightness from 0 to 100 (0—black colour, 100—white colour); the a* value—green-red colour; and the b* value—blue-yellow colour. The total colour difference (ΔE) is calculated according to the following equation:

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

2.8. Sensory Analysis

A sensory analysis of the jelly candies was carried out by 19 trained assessors, which consisted of trained students and staff of the Kaunas University of Technology (Kaunas, Lithuania) (7 males and 12 females, aged between 22 and 45), according to ISO 6658. Candy samples weighing 5 g were coded with three-digit random numbers and were served to the assessors on white plates in a monadic sequence. Water was provided for rinsing between the samples. The analysis was conducted at room temperature. Prior to the analysis, the samples were evaluated using the standard Descriptive Quantitative Analysis (DQA) [35] method for the selection of descriptors. Candies were evaluated for their apple aroma and taste, sourness, sweetness, and hardness using a 7-point intensity scale, where point 1 corresponded to the lowest intensity, and point 7 was corresponded to the highest intensity of the attribute. Candy samples were also evaluated for different attributes and overall acceptability using a 7-point hedonic scale, where point 1 was 'dislike extremely' and point 7 was 'like extremely'.

2.9. Statistical Analysis

All experiments were performed at least in triplicate. Mean values and standard deviations were calculated using MS Excel 2019 (Microsoft Corp., Albuquerque, NM, USA). A One-way analysis of variation (ANOVA), followed by Tukey's honest significant difference (HSD) test, was performed to determine significant differences ($p \leq 0.05$). Data were analysed using the Statgraphics Centurion 19 statistical package (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results

3.1. IDF, SDF, and RS Content in Enzymatically Treated Apple Pomace

The saccharide composition of enzymatically treated AP is presented in Figure 1. Low-molecular-weight carbohydrates, such as RS, composed a major part of the fraction of AP carbohydrates, while the DF content was significantly lower. Borujeni et al. [36] also reported a high sugar content with glucose (21.7%) and fructose (24.3%) as the primary reducing sugar in AP. According to the literature, the DF content in AP can range from 26.8 to 82.0%, and it depends on various factors, such as the plant variety, fruit maturity, and processing conditions [37].

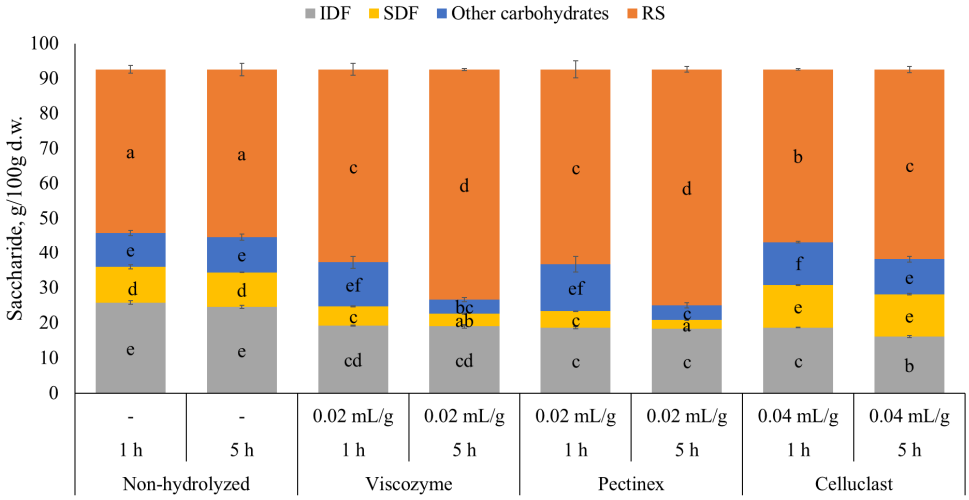


Figure 1. Saccharide composition of enzymatically hydrolysed AP. Data values are expressed as means with the standard deviation (n = 3). a–f means with different superscripts are significantly different ($p < 0.05$). IDF—insoluble dietary fibre; SDF—soluble dietary fibre; RS—reducing sugar.

The enzymatic hydrolysis significantly ($p \leq 0.05$) increased the RS content and decreased the TDF in all treated AP samples. The highest RS content was obtained after a 5 h hydrolysis with Viscozyme and Pectinex (1.38 and 1.4 times higher than the control, respectively). AP hydrolysis with the cellulolytic enzyme Celluclast increased the SDF content by approximately 20%; however, the duration of hydrolysis did not have a significant impact ($p \leq 0.05$) on the SDF content. Li et al. [38] also reported that cellulase increased the amount of SDF in AP after hydrolysis. Enzymatic hydrolysis with Pectinex and Viscozyme, which are composed of different carbohydrate mixtures, had a negative impact on IDF and SDF by reducing their content. It is known that pectin in the cell walls of apple is like protopectin, which is associated with cellulose and hemicellulose and reduces the effect of enzymes [39]. The synergistic effect of enzymes such as pectinases, cellulases, and β-glucosidases facilitates the breakdown of the pectin and lignin network and the access of the enzymes to the cellulose [40,41]. Note that in this study, the pectin of AP could be hydrolysed by pectinases from Viscozyme and Pectinex by increasing cellulase access to cellulose. While SDF could be hydrolysed to smaller fragments, i.e., oligosaccharides and mono- and disaccharides that are soluble in ethanol and are not identified as SDF, according to the AACC 32-07.01 and AOAC 991.43 methods, this may lead to an underestimation of the actual hydrolysis products and their potential benefits. Mrabet et al. [42] found a similar decrease in the TDF content of date fruit fibre after hydrolysis with Viscozyme. The obtained result indicates that AP, after enzymatic hydrolysis with Viscozyme and Pectinex, could be used as a value-added ingredient for fermentation or for sweet products as a natural sweetener, while AP hydrolysed with Celluclast could be used as an SDF source. However, AP is composed of a complex array of carbohydrates, and while enzymatic

hydrolysis using carbohydrase preparations such as Viscozyme, Pectinex, and Celluclast can be effective, its efficiency is limited by the accessibility of the substrate. The structural complexity of the cell wall and the interactions between its components may hinder enzymatic action, thus affecting the overall hydrolysis process and limiting the potential yield of functional ingredients.

3.2. Technological Properties of Enzymatically Treated Apple Pomace

Pomace as a dietary fibre source can be included in several food matrices as inexpensive substances that partially replace flour, fat, or sugar; as water and oil retention agents; and as substances that improve the stability of emulsions [43]. In order to use pomace in the production of food, it is important to evaluate its technological properties.

Pomace with a high water-retention capacity (WRC) and water-swelling capacity (WSC) could increase the thickness and stability of food products and could reduce syneresis. AP enzymatic hydrolysis with Viscozyme and Pectinex increased the WSC by 1.8 and 2.4 times, respectively, after 1 h of hydrolysis and 2.4 and 2.5 times after 5 h of hydrolysis (Figure 2a). Pectinex and Viscozyme, which contain several carbohydrases, may effectively degrade pectin and IDF, which leads to the formation of a higher amount of hydrogen bonds and an increase in the WSC [39,41].

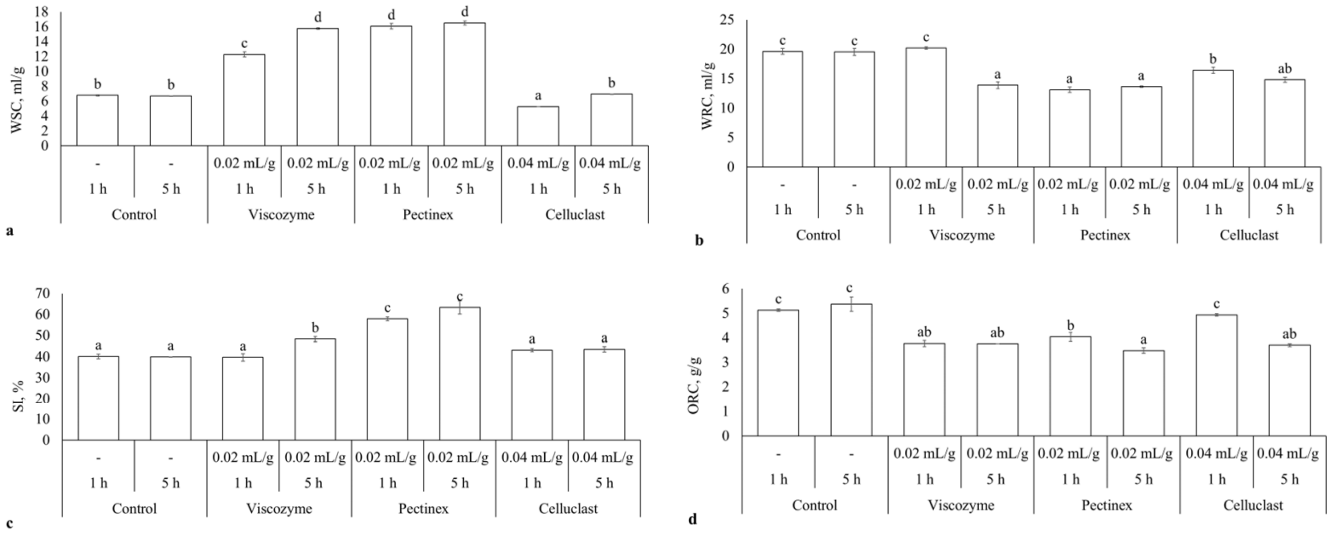


Figure 2. Water-swelling capacity (**a**), water-retention capacity (**b**), solubility index (**c**), and oil-retention capacity (**d**) of enzymatically hydrolysed AP. Data values are expressed as means with the standard deviation (n = 3). a–d means with different superscripts are significantly different ($p < 0.05$).

In this study, opposite trends were found after AP hydrolysis with Celluclast (0.04 mL/g), and the WSC decreased by 21.7% after 1 h of hydrolysis. During AP hydrolysis with cellulases, pectin is released from the matrix, whose properties of water swelling depend on the molecular weight and degree of esterification. Low-molecular-weight and low-methylation pectin is characterized by a lower WSC [44]. Dranca et al. [45] reported that AP hydrolysis with cellulases releases more low-methylation pectin [46].

The WRC of the enzymatically hydrolysed AP ranged from 13.14 to 20.21 g/g (Figure 2b). The lowest WRC value was determined after hydrolysis with Pectinex, which decreased by 33.13%. The ORC values of AP ranged from 3.70 to 5.38 g/g (Figure 2d). Hydrolysis with Pectinex had a negative effect on the ORC, after which the ORC decreased by 21.24 and 45.31% after 1 and 5 h of hydrolysis, respectively. The changes in the TDF and bulk density of the pomace (Figure 3) had a significant influence on the WRC and ORC. The technological properties of the pomace are closely related to the properties of

the material particles, which are reflected by the bulk density. A higher bulk density is associated with a lower porosity and particle size [47]. Hydrolysis with Viscozyme and Pectinex increased the AP bulk density. During the hydrolysis, the TDF content decreased by increasing the number of lower-molecular-weight compounds and the bulk density of the AP, indicating that the properties of the hydrolysed pomace particles changed [47], and reducing the matrix porosity does not retain bound water or oil under additional force (centrifugation) [43].

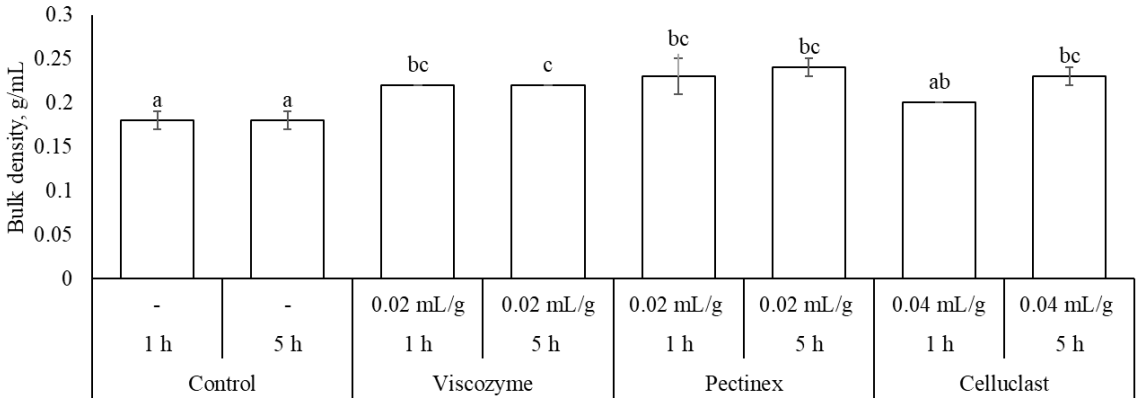


Figure 3. Bulk density of enzymatically hydrolysed AP. Data values are expressed as means with the standard deviation (n = 3). a–c means with different superscripts are significantly different ($p < 0.05$).

Hydrolysis for 5 h with Viscozyme increased the SI of the AP, while 1 h hydrolysis had no significant impact. The highest SI of AP was found after the hydrolysis with Pectinex (44 and 58% higher compared with the untreated AP after 1 and 5 h of hydrolysis, respectively). SI reflects the degradation of dietary fibre to form low-molecular-weight compounds. The hydrolysis of AP increased the content of soluble components (RSs) as well as SI values. Nemetz et al. [48] reported that the low solubility of pomace powder in water is caused by a high content of insoluble polysaccharides, their hydrophobicity, and electrostatic properties, which leads to the formation of aggregates and a decrease in solubility.

The influence of hydrolysed AP on the stability of emulsions under static and thermal conditions is presented in Figure 4. AP hydrolysed with Celluclast had lower emulsion stabilization properties than those hydrolysed with Pectinex and Viscozyme. AP hydrolysed with the Celluclast had a lower WSC value, while AP hydrolysed with the Viscozyme and Pectinex had higher WSCs; thus, the addition of the pomace hydrolysed with these enzymes could thicken the emulsion system more effectively, thus increasing its stability. Afrin et al. [49] reported that as the WSC of pomace increases, the stabilizing properties of their emulsions also increase.

During the preparation of various food products, heat treatment is often used; thus, it is important to evaluate the stability of emulsions after thermal treatment (80 °C). It is known that the stability of emulsions may decrease during heating depending on the presence of stabilizing agents [50,51]. Emulsions prepared with pomace enzymatically hydrolysed with Pectinex and Viscozyme, which had higher WSC values, were more stable. During heating, pomace can thicken the emulsion system, thereby increasing their stability [52].

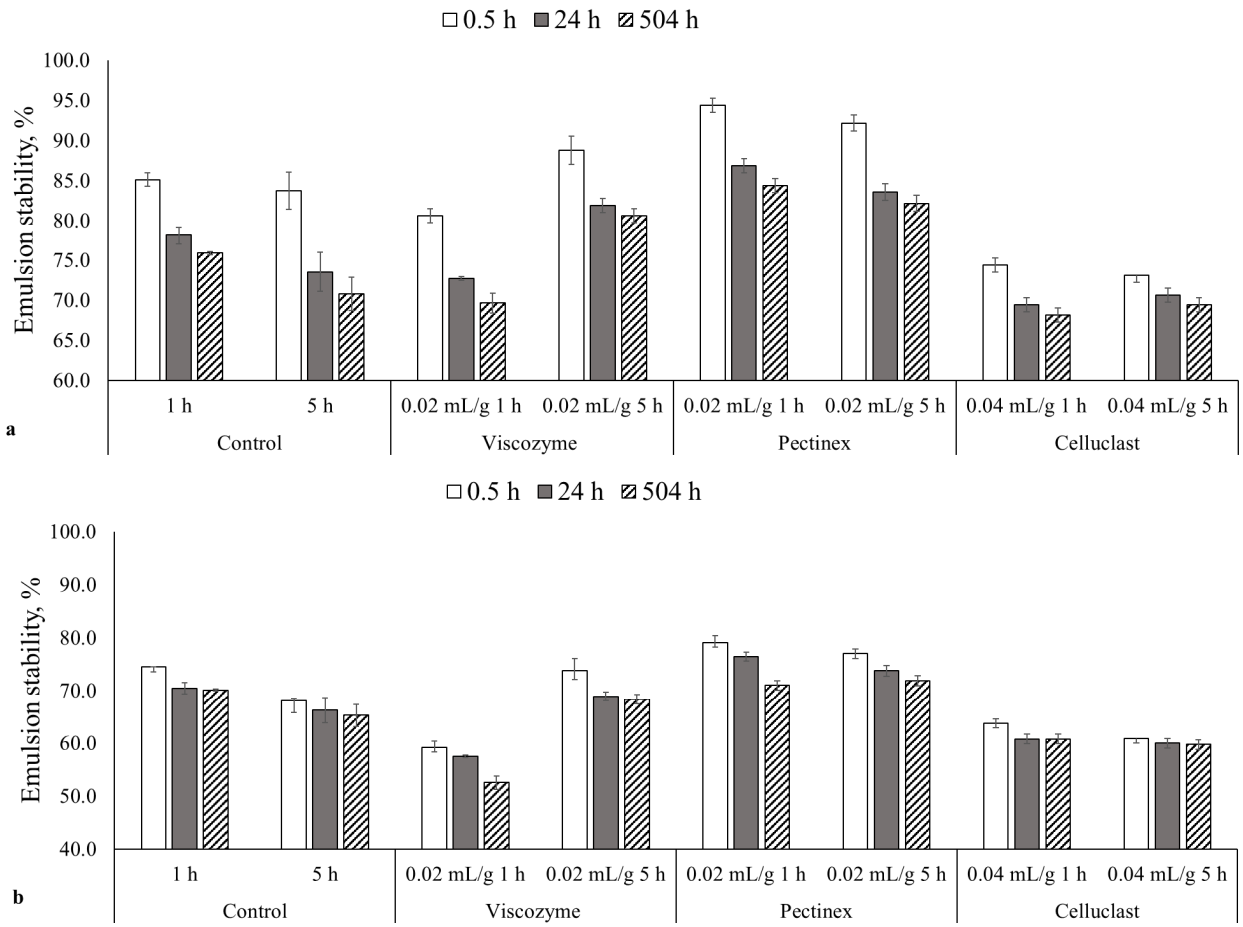


Figure 4. Influence of enzymatically hydrolysed AP on emulsion static (**a**) and thermal (**b**) stability during storage.

3.3. Jelly Candy Properties

AP hydrolysed with Celluclast was selected because of the highest SDF content obtained after hydrolysis and was used in the jelly candy recipe as a value-added ingredient. *Bifidobacterium animalis* DSM 20105 was chosen as a probiotic candidate which has a high tolerance to acidic environments (pH 2 and 3) and good autoaggregation and coaggregation properties [53]. Several studies have shown that *B. animalis* offers health benefits, such as enhancing gut health, lowering cholesterol (which is important for cardiovascular health), and modulating gut microbiota to help prevent conditions like constipation and diarrhoea. This modulation occurs through the production of short-chain fatty acids during fermentation, which may improve cholesterol metabolism and reduce inflammation. *B. animalis* has immunomodulatory effects, enhancing the immune response by promoting beneficial cytokines and inhibiting pro-inflammatory ones [54,55]. Additionally, *B. animalis* DSM 20105 has anti-inflammatory properties, as certain strains can modulate immune responses and decrease gut inflammation [56].

B. animalis DSM 20105 were incorporated into the AP matrix. The incorporation of probiotics into food matrices is frequently constrained by their viability during storage and their survival through the gastrointestinal tract. Therefore, the survival of probiotics in jelly candies during in vitro digestion, the viability of probiotics during storage, and candy quality characteristics were analysed.

3.3.1. Viability of Probiotics in Jelly Candies During Storage

The viability results of *B. animalis* DSM 20105 in jelly candy supplemented with AP (JCA) and candy without AP as a control (JCC) are presented in Table 1. The viability of *B. animalis* DSM 20105 decreased during storage in both products. Notwithstanding, after 56 days of storage, the viability of *B. animalis* DSM 20105 bacteria remained above 6 log10 CFU/g, which is recommended for probiotic food [57]. The higher bacterial viability during storage was observed in the JCA. The viability decreased by 1.22 log10 CFU/g and 1.52 log10 CFU/g in the JCA and JCC, respectively. Gelatine can be an excellent matrix for the immobilization of probiotic bacteria to ensure their longer viability, but the viability of the immobilized bacteria during storage depends on the storage conditions (temperature) and the additives used, such as various carbohydrates (prebiotics) [58]. Campaniello et al. [59] reported that *B. animalis* DSM 10140 exhibited better viability in a gelatine matrix than in alginate. Gelatine is often used to make an edible film supplemented with ingredients such as probiotics and prebiotics and to coat pieces of fruit (e.g., apples) [59]. The storage conditions and duration are the main factors responsible for the loss of viability of probiotic bacteria in this type of film, but films containing prebiotics retain bacterial viability longer [60].

Table 1. *B. animalis* DSM 20105 viability (log10 CFU/g) in jelly candies during storage at 4 °C.

Storage Days	JCC	JCA
1	7.80 ± 0.10^{aE}	7.86 ± 0.14^{aE}
7	7.48 ± 0.05^{aCD}	7.74 ± 0.08^{bDE}
14	7.35 ± 0.07^{aC}	7.63 ± 0.02^{bD}
28	6.80 ± 0.06^{aB}	7.38 ± 0.03^{bC}
42	6.69 ± 0.07^{aB}	7.11 ± 0.02^{bB}
56	6.28 ± 0.28^{aA}	6.64 ± 0.10^{aA}

Data values are expressed as means with the standard deviation (n = 3). JCC—control jelly candy without AP; JCA—jelly candy supplemented with AP. Values in one row that are followed by the same lowercase letter are not significantly different ($p \leq 0.05$), and the capital letters are not significantly different ($p \leq 0.05$) between samples in the column.

Several studies have shown that apple pomace carbohydrates such as pectin and oligosaccharides may have prebiotic potential. AP oligosaccharides have been shown to stimulate the growth of beneficial gut microorganisms, enhancing the efficacy of probiotics in food products [61]. Vlad et al. [62] also highlighted the significance of apple pomace's chemical composition, especially its pectin and polyphenols, making it suitable for incorporation into products. The SDF of pomace may also increase the viability of probiotics. Ibrahim et al. [63] reported that the protective effect of soluble fibres may be due to their ability to form a gel-like matrix, which shields probiotics from harsh environmental conditions, thereby enhancing their stability and viability.

3.3.2. Survival of Probiotics in Jelly Candies During In Vitro Digestion

The survival of *B. animalis* DSM 20105 in jelly candies during in vitro digestion was assessed at the end of storage (after 56 days), and the results are presented in Table 2. During digestion, *B. animalis* DSM 20105 viability in the gastric phase decreased by 0.13 log10 CFU/g in JCC and by 0.24 log10 CFU/g in JCA. After digestion in the intestinal phase, *B. animalis* DSM 20105 had a viability greater than 6 log10 CFU/g, which is the recommended amount for probiotic products [57]. The hydrolysed AP used for jelly candy supplementation did not increase the viability of the probiotic bacteria significantly during digestion. The immobilization of bacteria in various matrices, such as pectin or alginate, did not ensure greater bacterial viability during digestion, and the prebiotic substances used did not increase their viability [60,64]. Madureira et al. [65] reported that the viability

of *B. animalis* during in vitro digestion did not change significantly; however, viability may depend on both the strain used and the type of product. Meanwhile, Soares et al. [66] reported a reduced viability of *B. animalis* in orange juice during digestion in an acidic environment. Vlad et al. [62], in their study, demonstrated that apple pomace (rich in polysaccharides and polyphenolics) can exert a prebiotic effect that not only supports the growth of probiotics but also the survival of probiotics by facilitating their metabolic activities during digestion.

Table 2. *B. animalis* DSM 20105 viability (log10 CFU/g) in jelly candies during in vitro digestion.

Phases of In Vitro Digestion	JCC	JCA
Saline phase	6.28 ± 0.28^{aB}	6.64 ± 0.10^{aB}
Gastric phase	6.15 ± 0.14^{aB}	6.40 ± 0.11^{bB}
Intestine phase	5.79 ± 0.04^{aA}	6.09 ± 0.02^{bA}

Data values are expressed as means with the standard deviation (n = 3). JCC—control jelly candy without AP; JCA—jelly candy supplemented with AP. Values in one row followed by the same lowercase letter are not significantly different ($p \leq 0.05$), and the capital letters are not significantly different ($p \leq 0.05$) between samples in the column.

3.3.3. Quality Characteristics of Probiotic Jelly Candies

Changes in the acidity of jelly candies during storage are presented in Table 3. The pH of the probiotic jelly candies did not change significantly ($p < 0.05$) during storage, and jelly candies supplemented with AP had lower pH values and higher titratable acidity. Other authors reported that using *Lactobacillus acidophilus* La-5 reduces the pH values of this type of candy during storage [67].

Table 3. Changes in pH and TTA of jelly candies during storage at 4 °C.

Storage Days	pH		TTA, mL, 0.1 N of NaOH/10 g	
	JCC	JCA	JCC	JCA
1	4.78 ± 0.01^{aB}	4.56 ± 0.01^{aA}	5.17 ± 0.18^{aB}	6.55 ± 0.18^{aA}
7	4.79 ± 0.02^{aB}	4.56 ± 0.02^{aA}	5.12 ± 0.22^{aB}	6.57 ± 0.17^{aA}
14	4.78 ± 0.01^{aB}	4.56 ± 0.02^{aA}	5.18 ± 0.18^{aB}	6.55 ± 0.16^{aA}
28	4.80 ± 0.02^{aB}	4.60 ± 0.01^{aA}	5.12 ± 0.10^{aB}	6.57 ± 0.16^{aA}
42	4.80 ± 0.01^{aB}	4.58 ± 0.01^{aA}	5.16 ± 0.03^{aB}	6.50 ± 0.09^{aA}
56	4.80 ± 0.01^{aB}	4.58 ± 0.01^{aA}	5.15 ± 0.03^{aB}	6.58 ± 0.16^{aA}

Data values are expressed as means with the standard deviation (n = 3). JCC—control jelly candy without AP; JCA—jelly candy supplemented with AP; TTA—total titratable acidity. Values in one row followed by the same capital letter are not significantly different ($p \leq 0.05$), and the lowercase letters are not significantly different ($p \leq 0.05$) between samples in the column.

The hardness of the jelly candies (Table 4) was not significantly affected by the addition of hydrolysed AP at day 1, but after 56 days of storage, the hardness of the JCC significantly decreased, and the hardness of the JCA significantly increased. Changes in texture parameters are usually influenced by the structuring material and storage time [68]. Altınok et al. [69] reported that grape skin powder reduces the hardness of soft candy, which depends on the particle size and chemical composition of the additive. Other authors reported that the hardness of gelatine candies supplemented with white tea extracts increases during the first 2 months of storage [68]. The hardness of the JCC may have decreased due to the proteolytic activity of the probiotic bacteria, which weakened the gelatine structure. Candies supplemented with AP had a higher amount of SDF, including pectin, which increases the hardness of the material during storage [68]. The WRC of

pomace also contributes to textural properties, which depend on the interaction of various constituents and structural elements [69].

Table 4. Changes in hardness and colour characteristics of jelly candies during storage at 4 °C.

Candies	Storage Days	Hardness, N	Colour Characteristics			
			L*	a*	b*	ΔE
JCC	1	23.92 ± 1.06 ^{aA}	78.58 ± 0.33 ^{bD}	0.54 ± 0.02 ^{bD}	11.54 ± 0.47 ^{aB}	-
	56	36.39 ± 1.30 ^{bB}	73.63 ± 0.33 ^{aC}	−0.06 ± 0.04 ^{aC}	11.19 ± 0.32 ^{aB}	4.86 ± 0.35 ^{A}
JCA	1	24.49 ± 0.43 ^{aA}	37.20 ± 0.05 ^{bB}	9.53 ± 0.24 ^{bB}	12.73 ± 0.15 ^{bB}	-
	56	20.40 ± 0.84 ^{aA}	31.35 ± 0.11 ^{aA}	6.83 ± 0.34 ^{aA}	8.05 ± 0.33 ^{aA}	7.98 ± 0.33 ^{B}

Data values are expressed as means with the standard deviation (n = 3). JCC—control jelly candy without AP; JCA—jelly candy supplemented with AP. Values in one column followed by the same lowercase letter are not significantly different ($p \leq 0.05$) between the same jelly candies, and capital letters are not significantly different ($p \leq 0.05$) between all samples in the column.

The addition of pomace and the duration of storage had a significant effect on the colour characteristics of the products (Table 4). The pomace additive increased the darkness, redness, and yellowness of the product. However, these values changed significantly during storage in both products (ΔE > 2.5).

A sensory analysis is crucial when evaluating products, as it helps determine whether a new product will be acceptable to consumers and assesses the impact of added ingredients, which may influence and potentially limit the product's properties. The results of an evaluation of the sensory properties of the probiotic jelly candies are presented in Figure 5. JCA was characterized by a more intense sweetness, sourness, and apple aroma. The AP additive did not affect the hardness of the product. JCA was characterized by a more acceptable aroma, sourness, sweetness, and overall taste. M. Spinei [70] also reported that jelly candies supplemented with grape pomace was acceptable for assessors and did not have a negative impact on the candies' characteristics. These results indicate that AP could be used in jelly candies for improvements in nutritional value.

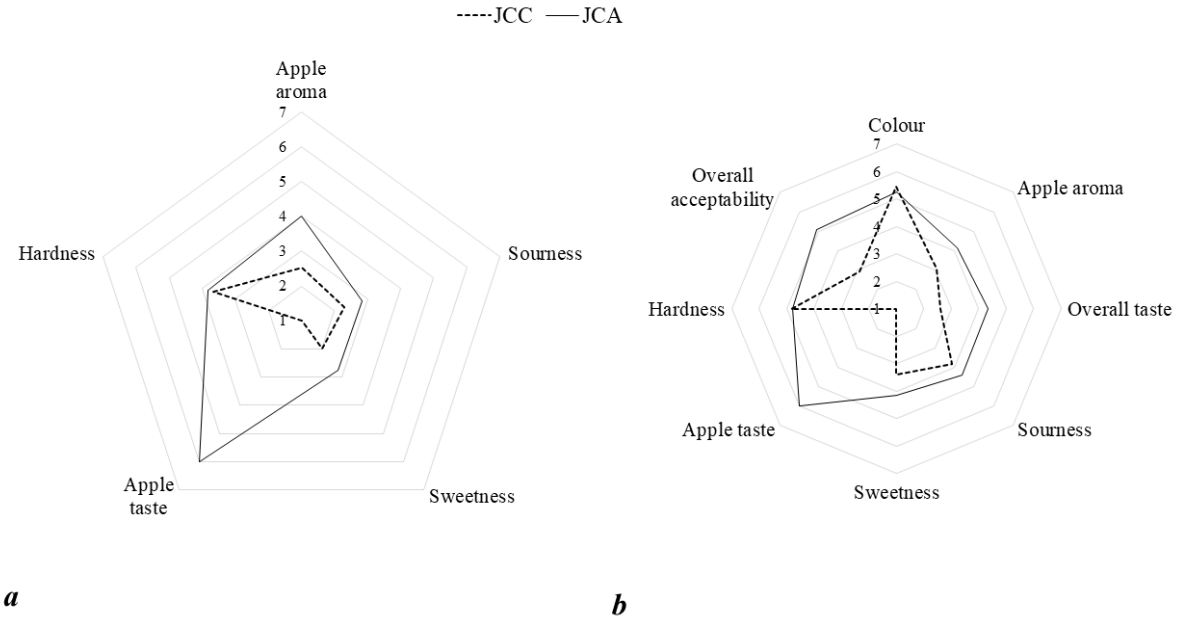


Figure 5. Effect of AP on jelly candy sensory properties: (**a**) intensity and (**b**) acceptability. JCC—control jelly candy without AP; JCA—jelly candy supplemented with AP.

Nevertheless, certain limitations of this study should be acknowledged. This study focused on the enzymatic hydrolysis of apple pomace using three commercial enzymes—Celluclast, Viscozyme, and Pectinex. However, using different enzyme combinations and different durations of an enzymatic hydrolysis treatment could yield distinct products and their technological properties. Further studies should focus on an evaluation of the impacts of other commercial enzymes on AP properties. In this study, the jelly candy production specifically focused on the application of AP enzymatically hydrolysed with Celluclast; therefore, the results might differ when other enzymes are used for hydrolysis. Altering the hydrolysis parameters (enzyme concentration, temperature, and duration) could result in different AP properties, consequently affecting the sensory characteristics of the jelly candies. In this study, gelatine was used as the main matrix for candies; using an alternative matrix could potentially yield different outcomes, particularly with regard to the viability of probiotics and the sensory characteristics of the product. Furthermore, only one probiotic strain was used in this study. Utilizing different probiotic strains could lead to varying results in terms of viability and survival during in vitro digestion and their overall effect on the product's properties. Future research involving different probiotic strains could help expand our understanding of their effects on product functionality and viability, though the current findings still provide a solid foundation for further investigation. Despite these limitations, the findings offer valuable insights for the future development of confectionery products supplemented with enzymatically hydrolysed AP and viable probiotic bacteria.

4. Conclusions

The enzymatically hydrolysed AP demonstrated different technological properties depending on the composition of the used commercial enzymes and the duration of the enzymatic hydrolysis. Hydrolysis with Pectinex® Ultra Tropical and Viscozyme® L significantly decreased the DF content by increasing the RS content, water-swelling capacity and water solubility index, and emulsion stability. Meanwhile, by using Celluclast® 1.5 L, the highest amount of SDF was produced. The probiotic *Bifidobacterium animalis* DSM 20105 viability in the jelly candies during 56 days of storage remained above the recommended level for probiotic food (more than 6 log10 CFU/g). A sensory evaluation indicated that the jelly candies enriched with hydrolysed AP and probiotics were acceptable to the assessors. The obtained results demonstrate that Celluclast® 1.5 L can be used for increasing the soluble dietary fibre in apple pomace, which can be further used in conjunction with *B. animalis* for added-value jelly candy production or can applied in other functional foods. These findings highlight the potential for pomace valorisation and its application in functional foods, not only by increasing its nutritional value and functionality as a probiotic carrier but also by showcasing its promise in the development of innovative food products. Further studies should focus on assessing the broader functional properties of this product and exploring the adaptability of such formulations using different types of pomaces. Additionally, studies should assess the viability of different probiotic strains, including their survival during storage and digestion. This could provide valuable insights into the potential applications and benefits of these products in various contexts.

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