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Towards the Valorization of Elderberry By-Product: Recovery and Use of Natural Ingredients for Sorbet Formulations

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Abstract: One of the food industry's greatest challenges is to find natural ingredients capable of conferring antioxidant and color properties. In addition, the agri-food industry generates by-products that are often treated as waste, despite their abundance of phytochemicals that can be recovered and used as food ingredients. This study explores the potential of elderberry pomace, an industrial by-product of juice processing rich in anthocyanins and polyphenols, as a natural food additive in blueberry sorbet. Elderberry pomace was incorporated into the sorbet formulation in powder form or as aqueous extracts at two different concentrations. The analysis of the pomace extract by UHPLC-DAD-MS showed the presence of four anthocyanins: cyanidin-3,5-*O*-diglucoside, cyanidin-3-*O*-sambubioside-5-*O*-glucoside, cyanidin-3-*O*-sambubioside, and cyanidin-3-*O*-glucoside. The physicochemical properties of the sorbets such as pH, °Brix, overrun, melting rate, and color were evaluated, as well as their levels of total phenolic compounds, total monomeric anthocyanins, and in vitro antioxidant activity. The potential of sorbets to stimulate the growth of probiotic bacteria was evaluated and a sensory analysis was conducted to assess consumer acceptance. Results indicated that the sorbet containing the more concentrated extract presented higher overrun, faster melting rate, higher contents of phenolic compounds and anthocyanins, and higher antioxidant activity compared to the control. Additionally, this formulation showed a darker hue (lower L^* value) and a tendency to stimulate probiotic bacteria. Moreover, the sorbets with pomace in their composition had good consumer acceptability. These findings highlight the potential of elderberry pomace to be used as a natural, sustainable ingredient in the ice cream industry, aligning with growing consumer trends towards healthier and eco-friendly products.

Keywords: elderberry; by-product; polyphenols; anthocyanins; recovery; sorbet; antioxidant; color; sensory properties

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1. Introduction

Ice creams and frozen desserts are very popular and appreciated by all age groups, not only during the summer for their refreshing sensation but also throughout the year. The food industry is currently conducting ongoing research to develop new goods in response to the needs of a consumer who is becoming more conscious of the impact of food on health and who is searching for wholesome and nourishing products without sacrificing their organoleptic qualities. In this regard, sorbets are healthier alternatives to ice cream and are appropriate for vegans, lactose intolerants, and consumers who intend to reduce their daily calorie intake [1]. Another great challenge for the food industry is the restriction of the use of some food additives, such as artificial food colorants and antioxidants, and the need to find natural ingredients capable of replacing them [2]. Fruits, vegetables, as well as their processing by-products are an interesting low-cost source of natural bioactive compounds and pigments [3]. Some works are described in the literature on the use of agro-industrial by-products as source of phenolic compounds and anthocyanins to enrich ice cream and giving it functional properties [4–7]. By repurposing these by-products, the food industry can minimize waste, create new revenue streams, and reduce dependency on synthetic additives, which often have a larger environmental footprint. This approach leverages the health benefits of natural ingredients, supports sustainable production practices, and fosters a circular economy, in which resources are continuously reused and environmental impact is minimized [8,9].

Berry fruits are well known for their high content in phenolic compounds, antioxidant capacity, and related health benefits. Particularly, when compared with other berries, elderberry (*Sambucus nigra* L.) is considered one of the best sources of anthocyanins and presents a powerful in vitro antioxidant activity [10]. Elderberry has long been associated with health benefits [11–13]. In addition to their high antioxidant potential [10,14–16], elderberry fruits are described as presenting anti-inflammatory [15,17], antidiabetic [18,19], and antiviral properties [20–22] and have a positive impact on obesity and metabolic disorders [23]. In addition to the previously mentioned beneficial health effects, recent studies have also shown that elderberry extracts have prebiotic properties [24–26]. This prebiotic effect may be due to the high content of polyphenolic compounds, since several studies have suggested a strong relationship between diets rich in polyphenols and the growth stimulation of probiotics [27,28].

Elderberry fruits are mostly processed into juices, concentrates, jams, syrups, and liqueurs [11]. The pomace, which is the main by-product resulting from the pressing of berries, can account for between 20 and 40% of total weight and has a high content of retained anthocyanins (75–98% of total anthocyanins) [29]. The dried pomace is also a good source of carbohydrates (82.4 g/100 g), calcium (164 mg/100 g), and magnesium (183 mg/100 g), and it has a low amount of fat (2.5 g/100 g) and may represent a potential alternative source of vegetal protein (5.9 g/100 g) and fiber (22.4 g/100 g) [30]. Despite its value, elderberry pomace is often used as fertilizer and in animal feed or treated as waste and few works explore its prospective use in the enrichment of food matrices [31–33]. For instance, the addition of elderberry pomace improved the nutritional value and organoleptic properties of shortbread cookies [32] and the pomace powder obtained from seedless fractions showed promising applicability as a coloring foodstuff in the preparation of yogurt [33]. Given the high anthocyanin content of elderberry pomace, it is a promising, underutilized resource for research in natural food colorants and antioxidants. Using elderberry by-products not only helps reduce waste and environmental impact but also aligns with the principles of a circular economy by turning processing waste into valuable ingredients. With its availability and nutritionally rich profile, elderberry pomace is a sustainable resource for innovative applications, from food enrichment to the development of natural additives, providing a robust foundation for further exploration in both research and industry.

Bearing all this in mind, the main purpose of this work was to help the ice cream industry with its need to find natural food colorants and stabilizers to improve the color and properties of a blueberry sorbet. For that, different blueberry sorbets were formulated with elderberry pomace, which was incorporated as (i) powder or (ii) aqueous extracts.

To evaluate the impact of adding pomace to sorbet, the following features were studied:

1. Physicochemical properties: Evaluation of the impact on sorbets' pH, °Brix, overrun, melting rate, and color.
2. Consumers' acceptance: Assessment of sensory characteristics of sorbets.
3. Bioactive content: Analysis of total phenolic content, total monomeric anthocyanins, and in vitro antioxidant potential.

4. Prebiotic potential: Evaluation of the ability of the sorbets enriched with pomace to stimulate the growth of probiotic bacteria.

This study represents an innovation by applying elderberry pomace specifically in blueberry sorbet, thereby expanding its usage beyond traditional applications in baked goods and dairy products. It introduces a dual incorporation method that utilizes both elderberry pomace powder or aqueous extracts, providing new insights into their effects on the sorbet's sensory and physicochemical properties. Emphasizing sustainability, this application aligns with trends toward natural ingredients within the ice cream sector.

2. Materials and Methods

2.1. Chemicals

Folin–Ciocalteu phenol reagent, methanol (analytical grade), and ethanol (analytical grade) were purchased from Fisher Scientific (Hampton, NH, USA). (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid 1-hydrate (99%), sodium carbonate anhydrous, sodium acetate anhydrous, and potassium chloride were obtained from Panreac (Barcelona, Spain).

2.2. Plant Raw Materials

Blueberries (*Vaccinium corymbosum*) were supplied by Acegrow Co. (Viseu, Portugal) and were immediately frozen at −18 °C.

Fresh elderberry pomace (*Sambucus nigra* L.) was kindly provided by a fruit juice factory (Indumape Co., Pombal, Portugal) and was stored at −18 °C until further use. The pomace was obtained after industrial production of juice from elderberry fruits harvested from plants cultivated in Varosa Valley in northern Portugal and were collected from three cultivars, "Sabugueira", "Bastardeira", and "Sabugueiro".

For better grinding on a laboratorial scale, the pomace was dried in a freeze-dryer (SP Scientific VirTis BenchTop Pro, Warminster, UK) in absence of light for 48 h and subsequently milled in a grinder (Qilive coffee grinder Q.5321, Auchan, Croix, France) for 2 min. After grinding, the pomace powder was packed in sterilized plastic flasks wrapped in aluminum foil and stored in a desiccator. Residual moisture content of pomace was recorded with a Mettler Toledo HG53 halogen moisture analyzer (Columbus, OH, USA).

2.3. Experimental Design

The sorbet formulation was developed in collaboration with a local company that prepares ingredients to produce artisanal ice cream (Beppo Gelados Co., Vila Nova de Paiva, Viseu, Portugal), aiming to find natural pigments and antioxidants that can be added to blueberry sorbet, improving its properties, without altering the sensory characteristics of the final product. All the sorbets were formulated to contain water, blueberries, sugar, blueberry syrup, and a neutral base composed of emulsifier and thickeners.

Figure 1 shows the main experimental steps for the preparation of the blueberry sorbets. Pomace was added to the sorbet formulations as an extract or a powder during a step of the syrup-making procedure. Overall, four different syrups were prepared according to their pomace content, giving rise to four different sorbets:

1. Sorbet C (control)—obtained using syrup C prepared only with blueberries, without addition of pomace powder or extract;
2. Sorbet P—obtained using syrup P (prepared with 5% pomace powder);
3. Sorbet E-5—obtained using syrup E-5 (prepared with 5% concentrated extract obtained from 5 g of pomace powder);
4. Sorbet E-50—obtained using syrup E-50 (prepared with 5% concentrated extract obtained from 50 g of pomace powder).

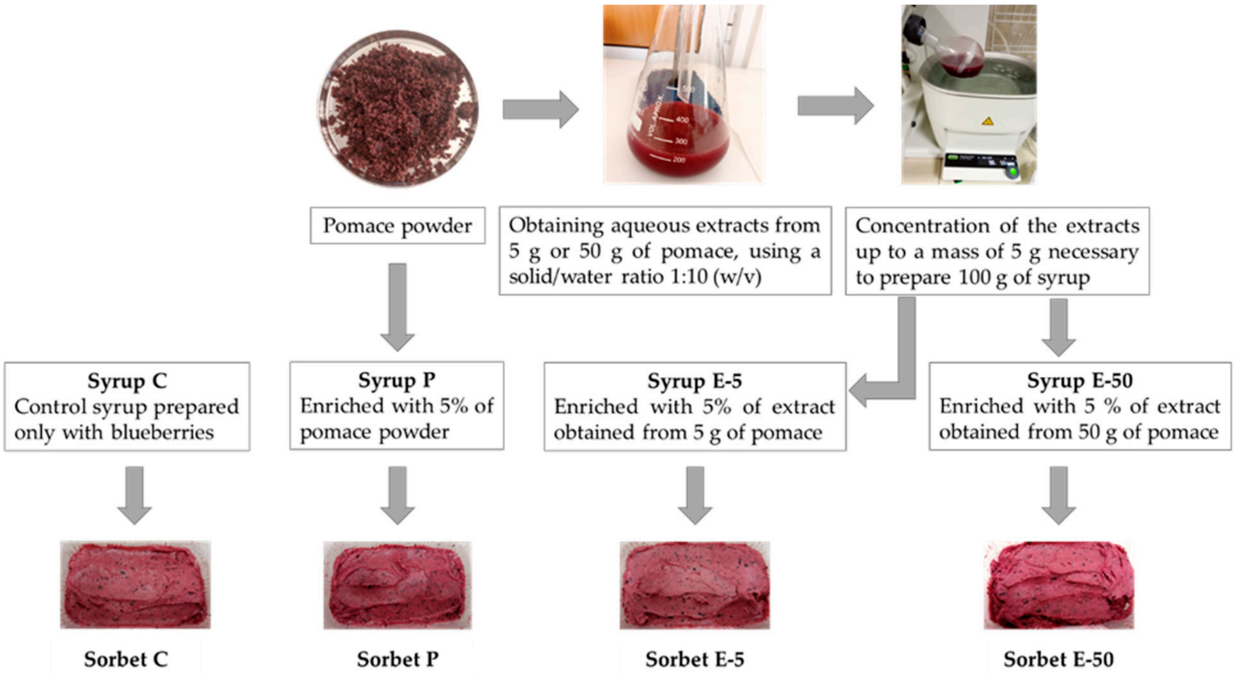

Figure 1. The main experimental steps for the preparation of the four sorbets.

2.4. Preparation of Elderberry Pomace Extracts

Two extracts from elderberry dried pomace powder (5 or 50 g) were obtained by conventional solvent extraction using water in a solid/solvent ratio 1:10 (*w*/*v*), under magnetic stirring (800 rpm) at room temperature (18 to 22 °C), for 30 min. The supernatants were passed through a glass funnel with cotton and then through filter paper (filtraTECH, QL01, Saint-Jean-de-Braye, France, pore size > 20 µm) using a Büchner funnel. The remaining solids were reextracted, and filtrates were combined. The water of each extract was removed using a rotary evaporator (BÜCHI Rotavapor R-300, New Castle, DE, USA) and vacuum pump (BÜCHI Vacuum Pump V-300) until obtaining a final mass of 5 g of extract plus water. In this way, after evaporation of water, the extract obtained with 50 g of pomace was 10 times more concentrated in recovered compounds than that obtained from 5 g. The final extracts were subsequently used in the preparation of blueberry syrups needed for the formulation of sorbets E-5 and E-50. To characterize elderberry pomace, extracts were prepared as previously described and total phenolic compounds, total monomeric anthocyanins, the $ABTS^{\bullet+}$ antioxidant capacity, and the main individual phenolic compounds according to HPLC-DAD-ESI-MS were analyzed.

2.5. Analysis of Pomace Polyphenols Using Ultra-High-Performance Liquid Chromatography with Photodiode Array Detector and Mass Spectrometry (UHPLC-DAD-ESI-MS^n)

The UHPLC-DAD-ESI-MS analysis was conducted using an Ultimate 3000 system (Dionex Co., San Jose, CA, USA) comprising an auto-sampler, quaternary pump, diode array detector, and automated thermostatic column compartment (Ultimate 3000, Dionex, San Jose, CA, USA). This system was connected to an ion trap mass spectrometer with an electrospray ionization (ESI) source (Thermo LTQ XL MS, Thermo Scientific, San Jose, CA, USA). ESI needle voltage was set to 4.80 kV, the capillary temperature was maintained at 275 °C, and high-purity nitrogen gas (>99%) was used at 520 kPa. The device operated in positive-ion mode, scanning over an m/z range from 100 to 2000. Data acquisition and system control were performed using a Thermo Xcalibur Qual Browser (Thermo Scientific, San Jose, CA, USA).

Compounds were separated on a Hypersil GOLD C18 column (100 mm length, 2.1 mm internal diameter, 1.9 µm particle size; Thermo Scientific, Waltham, MA, USA), with temperature set to 30 °C. Gradient elution was performed using eluent A (0.1% formic acid in water) and eluent B (30% methanol in acetonitrile) at a flow rate of 0.200 mL/min. The gradient program started with 5% eluent B, increased to 40% over 14 min, then ramped up to 100% at 16 min, holding for 2 min before returning to the initial conditions by 20 min [34,35]. UV–Vis spectral data for all peaks were recorded over the 200–700 nm range, with chromatographic profiles monitored at 280 and 520 nm.

2.6. Preparation of Blueberry Syrup Enriched with Elderberry Pomace Powder or Extract

The composition of the blueberry syrups consisted of the following ingredients: 16.0% frozen blueberries, 65.4% commercial sugar, 1.2% pectin, 5.0% elderberry pomace powder or liquid pomace extract, and 12.4% water (*w*/*w* basis). All the ingredients were weighed separately and then mixed in a cooking pot where they were subjected to heating in an induction cooker at medium temperature for 6 min with continuous manual agitation with a spoon. After cooling, the syrups were transferred to sterilized plastic bottles and kept at room temperature, protected from light. The blueberry syrup used as control was prepared by replacing the pomace powder or extract with the same mass of water.

2.7. Preparation of Sorbet

The sorbet mixtures were formulated to contain 52.8% water, 22.6% frozen blueberries, 18.8% commercial sugar, 0.5% neutral base composed of emulsifier and thickeners (cremodan 500 coldline, provided by Beppo, Makati, Philippines), and 5.3% blueberry syrup (*w*/*w* basis). All the ingredients were weighed separately, according to the sorbet formulation (Section 2.3). The ingredients were incorporated, and then the mixture was homogenized for 2 min in a liquefier. The mixture was further transferred to an ice cream machine (CUBE 750 Professional Ice Cream Maker, Montebello della Battaglia, Pavia, Italy) and was frozen for 20 min with agitation. The control sample was also made following the same procedure as described above. The sorbets were packaged in plastic containers and stored at −18 °C. For each sorbet, two replicates were carried out.

2.8. Quality Evaluation of Sorbets

2.8.1. pH of Sorbet

The pH of the samples was measured directly using a Consort C1010 multi-parameter pH analyzer (Turnhout, Belgium). About 30 mL of the sorbet sample was poured into a 50 mL beaker, allowed to melt at room temperature for 1 h, and then the electrode was inserted while the sample was gently agitated. The samples were analyzed in triplicate.

2.8.2. °Brix of Sorbet

The sorbet samples (about 30 mL) were allowed to melt at room temperature for 1 h and then the °Brix of the samples was read on a digital refractometer (ATAGO USA Inc., Belleuve, WA, USA) without the need for dilution. The auto-zero was carried out with distilled water and each analysis was performed in triplicate.

2.8.3. Overrun

Overrun measurement was taken per sample by comparing the weight of sorbet mix before freezing and sorbet after freezing in a fixed-volume container (30 mL). Overrun (%) was calculated as follows:

$$\text{Overrun (\%)} = \frac{\text{Sample weight before freezing} - \text{Sample weight after freezing}}{\text{Sample weight after freezing}} \times 100 \quad (1)$$

2.8.4. Melting Rate

For the meltdown tests, samples of each sorbet were collected with a cooled metal ring in the form of a cone (volume of 30 mL) from the containers stored at −18 °C [36]. The samples were weighed and placed on a wire mesh (wire thickness 2 mm) on the top of a funnel that was attached to a beaker on a balance at a controlled temperature of

20 ± 2 °C [37,38]. The mass of melted sorbet passing through the funnel was recorded every 5 min for 60 min. The melting curves were plotted as the percentage of the sorbet's original mass as a function of time [38]. All the samples were analyzed in triplicate.

2.8.5. Color Analysis

Color attributes of sorbets were measured using a digital chromameter (Konica Minolta CR-400, Tokyo, Japan), operating in diffusion illumination 0° viewing angle geometry, specular component included (SCI) mode, using D65 illuminant, and expressed in the CIE $L^*a^*b^*$ system where L^*—lightness of sorbet (1–100), a^*—redness (+100) and greenness (−100), b^*—yellowness (+100) and blueness (−100). The color of sorbets was also evaluated in terms of the total color difference (ΔE) in relation to the control sorbet C, calculated according to Equation (2):

$$\Delta E = \sqrt{\left(L^*_{\text{sample}} - L^*_{\text{control}}\right)^2 + \left(a^*_{\text{sample}} - a^*_{\text{control}}\right)^2 + \left(b^*_{\text{sample}} - b^*_{\text{control}}\right)^2} \quad (2)$$

The colorimeter was calibrated against a standard instrument white tile (L = 97.80, a = −0.08, b = 1.82). All the samples were analyzed in triplicate.

2.8.6. Consumer Evaluation of Sorbets

For the consumer evaluation, 1.5 kg of each sorbet was prepared and stored at −18 °C for 24 h. On the day of analysis, the sorbets were left for 5 min at 20 ± 2 °C before serving [6]. A total of 43 panelists were recruited from the academic population at the Agrarian School of the Polytechnic University of Viseu, Portugal with ages ranging between 18 and 63. Samples were served as 70 mL in a paper cup and analyzed at 20 ± 2 °C with a clear view under white lights. A glass of water was provided for palate cleansing. Consumer acceptance was evaluated for color, creaminess, coldness, flavor, taste sensation, sweetness, acidity, and global appreciation using a hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) [39]. Panelists were also asked to choose one sorbet according to their consumer preference.

2.9. Preparation of Extracts from Sorbets for Phenolics and Antioxidant Activity Analysis

Preparation of extracts from sorbets was carried out according to Akca and Akpinar with some modifications [40]. To obtain the extracts, 2 g of sorbet was dissolved in 10 mL of methanol and mixed for 5 min in a magnetic stirrer (800 rpm) at room temperature. The mixture was then centrifuged for 10 min at 1409× g at 20 °C (Hettich UNIVERSAL 16, Tuttlingen, Germany). The supernatant was separated and used as a crude extract to determine total phenolic content, total monomeric anthocyanins, and $ABTS^{\bullet+}$ antioxidant capacity.

2.10. Determination of Total Phenolic Content (TPC)

The total phenolic content in pomace and sorbet extracts was quantified using the Folin–Ciocalteu method with gallic acid as a standard, as outlined in our previous work [16]. To prepare each sample, 125 µL of Folin–Ciocalteu reagent was combined with 125 µL of sample (extract or standard solution) and 1 mL of distilled water in test tubes. This mixture was vortexed, allowed to stand for 6 min at room temperature in the dark, and then combined with 2 mL of sodium carbonate solution 5% (w/v). The samples were then homogenized by vortexing and incubated in the dark at room temperature for 60 min. After the designated time, samples were promptly transferred to 1 cm polystyrene cuvettes, and absorbance was recorded at 760 nm in triplicate using a UV–visible spectrophotometer (Shimadzu UV-1280, Izasa Scientific, Barcelona, Spain). The total phenolic content was calculated in triplicate based on a standard curve of gallic acid and expressed as milligrams of gallic acid equivalents per gram (mg GAE g^{-1}) of dried pomace or per kilogram (mg GAE kg^{-1}) of sorbet.

2.11. Determination of Total Monomeric Anthocyanins (TMAs)

The total monomeric anthocyanin content in pomace and sorbet extracts was measured using the pH differential method, following the procedure described by Giusti and Wrolstad [16,41,42]. To establish the appropriate dilution factor (*DF*) for analysis, extracts were first diluted with pH 1 potassium chloride buffer until the absorbance at 510 nm was below 1.2. For the anthocyanin quantification assay, each sample was then diluted separately with potassium chloride buffer (pH 1) and potassium acetate buffer (pH 4.5) using this predetermined dilution factor. The mixtures were left in the dark for 15 min, after which absorbance of each buffer-prepared sample was measured at 510 and 700 nm in triplicate using 1 cm polystyrene cuvettes and a UV–visible spectrophotometer (Shimadzu UV-1280, Izasa Scientific, Barcelona, Spain).

The concentration of total monomeric anthocyanins (mg L^{-1}) was calculated using Equation (3):

$$\text{TMA}\left(\text{mg L}^{-1}\right) = \frac{A \times MW \times DF}{\varepsilon \times l} \times 1000 \quad (3)$$

where MW is the molecular mass of cyanidin-3-*O*-glucoside (449.2 g mol^{-1}), DF is the dilution factor, ε is the molar absorptivity coefficient of cyanidin-3-*O*-glucoside (26,900 L mol^{-1} cm^{-1}), l is optical path length in cm, and A is calculated as follows:

$$A = \left[(A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH1}} - (A_{510\text{ nm}} - A_{700\text{ nm}})_{\text{pH4.5}}\right] \quad (4)$$

Results are reported as milligrams of cyanidin-3-*O*-glucoside equivalents per gram (mg cy3glcE g^{-1}) of dried pomace or per kilogram (mg cy3glcE kg^{-1}) of sorbet.

2.12. Determination of Antioxidant Activity

The antioxidant activity of pomace and sorbet extracts was assessed by the $ABTS^{\bullet+}$-scavenging method, based on a previously described protocol [43], and which has also been applied in elderberry matrices [16,44]. $ABTS^{\bullet+}$ was generated by dissolving ABTS with a 2.45 mM aqueous solution of potassium persulfate to a final concentration of 7 mM ABTS. To ensure complete conversion, the mixture was allowed to stand in the dark at room temperature for 16 h. It was then transferred to polypropylene tubes and stored at −18 °C until required. On the day of analysis, the $ABTS^{\bullet+}$ solution was further diluted with ethanol to adjust the absorbance to 0.700 at 734 nm. For the assays, the extracts were previously diluted 1:5 in methanol, and then 100 µL of each diluted extract was combined with 2 mL of the prepared $ABTS^{\bullet+}$ solution in test tubes. The mixtures were homogenized using a vortex and left in the dark at room temperature for 15 min. Absorbance readings were taken in triplicate at 734 nm using 1 cm polystyrene cuvettes and a UV–visible spectrophotometer (Shimadzu UV-1280, Izasa Scientific, Barcelona, Spain). A blank was also prepared by replacing the 100 µL of extract with methanol. The percentage inhibition of $ABTS^{\bullet+}$ was calculated using the following equation:

$$\%\ \text{ABTS}^{\bullet+}\ \text{inhibition} = \frac{A_{(\text{blank solution})} - A_{(\text{sample})}}{A_{(\text{blank solution})}} \times 100 \quad (5)$$

The antioxidant activity of pomace or sorbet extracts was expressed in mmol Trolox equivalents per gram (mmol TE g^{-1}) of dried pomace or per kilogram (mmol TE kg^{-1}) of sorbet, based on a Trolox calibration curve.

2.13. Sources and Cultivation of Probiotic Cultures

The bacterial inoculum composed of *Lactobacillus acidophilus* La-5, *Bifidobacterium* BB-12, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains was previously prepared in an MRS medium for 48 h at 37 °C. Fresh cultures of the probiotic bacteria were added to sterile MRS media at a ratio of 1% (inoculum volume/final volume of culture medium) containing 1% of sorbet (sorbet mass/final volume) and incubated at 37 °C. Simultaneously, a test was carried out under the same conditions without the addition of any sorbet (negative control). The pH of the growth medium for each assay was measured in triplicate at 0, 3, 8, 24, and 48 h and aliquots were taken at 0, 8, and 24 h and inoculated onto plates using MRS culture medium for counting of viable colonies (log CFU/mL) after 72 h of incubation, from which the mean values and standard deviation were calculated.

2.14. Statistical Analysis

The experimental data were statistically analyzed using the IBM SPSS Statistics software, version 28.0.0.0 (Chicago, IL, USA). Analysis of variances was performed through a Kruskal–Wallis one-way ANOVA followed by the Dunn's post hoc test with a Bonferroni adjustment. Differences among the groups were determined as statistically significant at a level of $p < 0.05$.

3. Results and Discussion

3.1. Characterization of Elderberry Pomace

Elderberry pomace after freeze-drying presented an average dry mass content of 99.54 ± 0.03%. Solvent extraction with water, after two sequential extractions, allowed us to obtain a total phenolic content of 2.98 mg GAE g^{-1} of pomace, 1.48 mg cy3glcE g^{-1} of total monomeric anthocyanins, and $ABTS^{\bullet+}$-scavenging activity of 21.10 mmol TE g^{-1} (Table 1).

Table 1. Content of total phenolic content (TPC), total monomeric anthocyanins (TMAs), and $ABTS^{\bullet+}$-scavenging activity of elderberry pomace extract, expressed per gram of dry pomace [1].

TPC (mg GAE g^{-1})	TMAs (mg cy3glcE g^{-1})	$ABTS^{\bullet+}$ (mmol TE g^{-1})
2.98 ± 0.45	1.48 ± 0.18	21.10 ± 2.29

[1] GAE—gallic acid equivalents, cy3glcE—cyanidin-3-*O*-glucoside equivalents, TE—Trolox equivalents. Results are expressed as mean values ± standard deviation. The analyses consisted of two replicates and were performed in triplicate.

The UHPLC-DAD-MS analysis of the extract with detection in a range of 200–700 nm exhibited two peaks with typical anthocyanin absorption spectra (λ_{max} at 280 and 518 nm). Four anthocyanins have been identified based on their $[M]^+$ ions and on their MS^2 spectra (Figure 2). Compounds 1 and 2 eluting at about 6.8 min were identified as cyanidin-3,5-*O*-diglucoside ($[M]^+$ at m/z 611, product ions at m/z 449, 287) and cyanidin-3-*O*-sambubioside-5-*O*-glucoside ($[M]^+$ at m/z 743, product ions at m/z 581, 449, 287). Compounds 3 and 4 eluting at about 9.0 min were identified as cyanidin-3-*O*-sambubioside ($[M]^+$ at m/z 581, product ion at m/z 287) and cyanidin-3-*O*-glucoside ($[M]^+$ at m/z 449, product ion at m/z 287). These results are in accordance with literature data [45–47]. Although elderberry is rich in quercetin-3-*O*-glucoside and rutin, these derivatives were not found in the pomace extract, probably due to their low solubility in water.

3.2. Evaluation of Physicochemical Parameters of Sorbets

Table 2 presents some physicochemical parameters of the sorbets prepared. All the sorbets have an acidic pH (3.51–3.68) which is characteristic of blueberry fruits. The sorbet that was prepared with the syrup that contained the most concentrated pomace extract (E-50) is slightly less acidic (pH = 3.68 ± 0.06). The °Brix value is also similar in the various sorbets with values between 25.90 and 26.30.

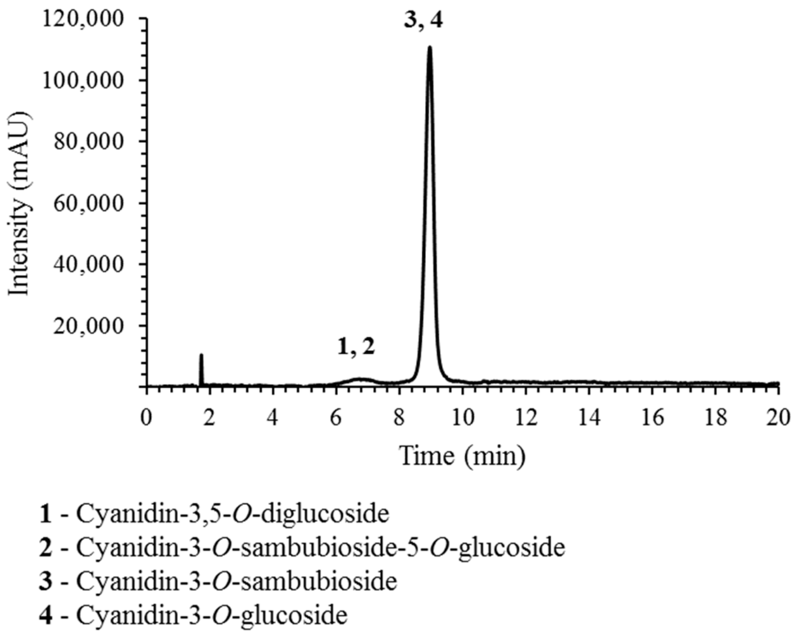

Figure 2. UHPLC chromatogram of elderberry pomace extract recorded at 520 nm.

Table 2. pH, °Brix, and overrun values of prepared sorbets [1].

Sorbet	pH [2]	°Brix [2]	Overrun (%)
C	3.52 ± 0.02 [b]	26.25 ± 0.08 [a,b]	13.7
P	3.51 ± 0.05 [b]	25.90 ± 0.25 [b]	28.3
E-5	3.52 ± 0.01 [b]	26.22 ± 0.08 [a,b]	10.9
E-50	3.68 ± 0.05 [a]	26.30 ± 0.09 [a]	19.0

[1] C—control sorbet prepared without addition of pomace powder or extract; P—obtained using syrup P prepared with 5% pomace powder; E-5—obtained using syrup E-5 prepared with 5% concentrated extract obtained from 5 g of pomace powder; E-50—obtained using syrup E-50 prepared with 5% concentrated extract obtained from 50 g of pomace powder. [2] Results are expressed as mean values ± standard deviation. The analyses consisted of two replicates and were performed in triplicate. Means followed by the same letter within a column are not significantly different ($p < 0.05$).

The overrun is related to the volume and amount of air present in the sorbet and is one of the main criteria for evaluating the quality and profitability of the product [38]. The overrun depends on several factors, such as the presence of fat, sweeteners, and stabilizers. Sorbet P, which was prepared with the addition of powdered pomace, had the highest overrun value (28.3%), followed by sorbet E-50 (19.0%), which contains the most concentrated pomace extract, while sorbet C without added pomace and sorbet E-5 prepared with the most diluted pomace extract showed lower values, 13.7 and 10.9%, respectively.

The melting rate of ice cream and other frozen desserts is influenced by several factors, including overrun, the amount of air incorporated, the nature of the ice crystals, and the extent of fat destabilization [48]. The sorbet melting curves are shown in Figure 3. Sorbet E-50 melts faster than the other sorbets. Sorbets E-5 and P present similar melting curves and melt more slowly than sorbet C (sorbet without added pomace), however, sorbet P has a behavior very close to sorbet C in the first 40 min. Sorbets P and E-5 melt more slowly than sorbet C.

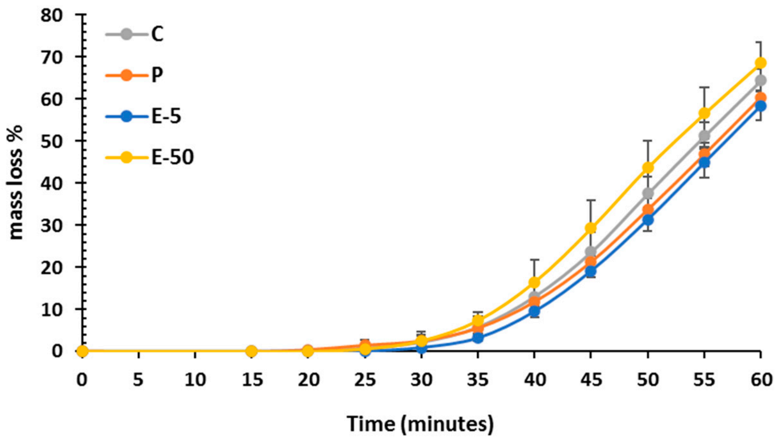

Figure 3. Sorbet melting rate as a function of time. Data correspond to mean values and the vertical bars correspond to the standard deviations.

The sorbet color attributes are presented in Table 3. The *L** parameter presented values between 39.4 and 47.5. Compared with the sorbet obtained without adding pomace (sorbet C), sorbet E-50, which contains concentrated elderberry pomace extract, exhibited lower values of the parameter *L**, indicating that the addition of pomace contributed to darker color attributes in sorbet. The lower values of *L** in this sample are related to the presence of a higher content of anthocyanins that give a darker hue to the sorbet. The *a** values are between 25.1 and 27.3 and the *b** values between 8.0 and 11.5. Sorbet E-50 that contains the most concentrated pomace extract in its composition showed a tendency toward a lower *b** value. Although this difference was not statistically significant, it is consistent with a higher anthocyanin content, contributing to a bluer hue in the final product.

Table 3. Color attributes of the sorbets [1].

Sorbet	*L**	*a**	*b**	ΔE
C	46.1 ± 4.6 [a]	27.3 ± 2.3 [a]	10.3 ± 1.0 [a,b]	-
P	41.4 ± 4.4 [a,b]	27.3 ± 2.4 [a]	10.6 ± 2.0 [a,b]	7.0 ± 2.7 [a,b]
E-5	47.5 ± 6.1 [a,b]	26.9 ± 1.6 [a]	11.5 ± 1.0 [a]	3.9 ± 1.8 [b]
E-50	39.4 ± 1.1 [b]	25.1 ± 3.3 [a]	8.0 ± 1.0 [b]	9.3 ± 3.9 [a]

[1] *L**—lightness (1–100), *a**—redness (+100) and greenness (−100), *b**—yellowness (+100) and blueness (−100). Results are expressed as mean ± standard deviation. The analyses consisted of two replicates and were performed in triplicate. Means followed by the same letter within a column are not significantly different ($p < 0.05$).

3.3. Consumer Assessments of Sorbets

In the various attributes evaluated, all the sorbets showed high acceptability by the tasters with an average score of 5.7 to 7.7 (Table 4). No significant differences were found in the appreciation of the different attributes in sorbets containing elderberry pomace in relation to the sorbet obtained without adding pomace (sorbet C), which indicates that the addition of pomace did not change the sensory attributes of the sorbet. When asked to select a sorbet based on their consumer preference, the C and E-50 sorbets were the most favored, receiving 37.5% and 27.5% of the votes, respectively. Interestingly, 62.5% of participants expressed a preference for sorbets that included elderberry pomace in their formulation.

Table 4. Results of consumer evaluation of sorbets [1].

Sorbet	Visual Appearance	Creaminess	Coldness	Flavor	Taste Sensation	Sweetness	Acidity	Global Appreciation	Consumer Choice (%)
C	7.6 ± 1.2 [a]	7.0 ± 1.6 [a]	7.0 ± 1.4 [a]	6.8 ± 1.5 [a]	6.9 ± 1.7 [a]	6.6 ± 1.9 [a]	6.0 ± 2.0 [a]	7.2 ± 1.7 [a]	37.5
P	7.6 ± 0.9 [a]	6.7 ± 1.6 [a]	7.0 ± 1.6 [a]	6.4 ± 1.9 [a]	6.4 ± 1.6 [a]	5.8 ± 2.1 [a]	5.9 ± 1.9 [a]	6.3 ± 1.7 [a]	10.0
E-5	7.5 ± 1.2 [a]	6.9 ± 1.6 [a]	7.1 ± 1.4 [a]	6.4 ± 1.6 [a]	6.5 ± 1.9 [a]	5.9 ± 2.2 [a]	5.8 ± 2.1 [a]	6.8 ± 1.8 [a]	25.0
E-50	7.7 ± 1.0 [a]	6.8 ± 1.8 [a]	6.8 ± 1.4 [a]	6.7 ± 1.9 [a]	6.6 ± 1.9 [a]	6.5 ± 2.2 [a]	5.7 ± 2.1 [a]	6.8 ± 1.8 [a]	27.5

[1] Results are expressed as mean values ± standard deviation (n = 43). Means followed by the same letter within a column are not significantly different ($p < 0.05$).

3.4. Antioxidant Potential of Sorbets

The sorbets were analyzed for the content of total phenolic compounds, monomeric anthocyanins, and in vitro antioxidant activity in relation to the $ABTS^{\bullet+}$ cation radical (Table 5). The total phenolic compound content of sorbets ranged from 561.04 to 665.03 mg GAE kg^{-1} of sorbet, the value of monomeric anthocyanins ranged from 196.20 to 259.29 mg cy3glcE kg^{-1} of sorbet, and the antioxidant activity against the $ABTS^{\bullet+}$ cation radical between 3.84 and 4.39 mmol TE kg^{-1} of sorbet. The sorbets that contain elderberry pomace in their composition show higher values of the bioactive compounds analyzed as well as higher antioxidant activity compared to sorbet C (without the addition of pomace). The highest values of bioactive compounds and antioxidant activity belong to sorbet E-50 that was prepared with the most concentrated elderberry pomace extract (665.03 mg GAE kg^{-1}, 259.29 mg cy3glcE kg^{-1}, and 4.39 mmol TE kg^{-1}, respectively, for total phenolic compounds, monomeric anthocyanins, and $ABTS^{\bullet+}$ antioxidant activity).

Table 5. Contents of total phenolic content (TPC), total monomeric anthocyanins (TMAs), and $ABTS^{\bullet+}$ scavenging activity of sorbets [1].

Sorbet	TPC (mg GAE kg^{-1})	TMAs (mg cy3glcE kg^{-1})	$ABTS^{\bullet+}$-Scavenging Activity (mmol TE kg^{-1})
C	561.04 ± 7.93 [b]	196.20 ± 2.60 [b]	3.84 ± 0.07 [b]
P	643.38 ± 21.20 [a,b]	223.27 ± 5.10 [a,b]	4.20 ± 0.08 [a,b]
E-5	655.60 ± 7.30 [a,b]	249.43 ± 17.63 [a]	4.23 ± 0.10 [a,b]
E-50	665.03 ± 15.38 [a]	259.29 ± 2.80 [a]	4.39 ± 0.07 [a]

[1] GAE—gallic acid equivalents, cy3glcE—cyanidin-3-*O*-glucoside equivalents, TE—Trolox equivalents. Results are expressed as mean values ± standard deviation. The analyses consisted of two replicates and were performed in triplicate. Means followed by the same letter within a column are not significantly different ($p < 0.05$).

3.5. Evaluation of the In Vitro Prebiotic Effect of Blueberry Sorbet

The pH of inoculated growth media was measured at 0, 3, 8, 24, and 48 h and the bacterial growth expressed as log CFU/mL was calculated at 0, 8, and 24 h (Tables 6 and 7, respectively). The results of the pH analysis presented in Table 6 show a decrease in pH in all culture media as a result of lactic acid production. In media containing blueberry sorbet there is a greater reduction in pH (from 6.12–6.20 to 4.57–4.62) when compared to the medium without the addition of sorbet (negative control, from 6.24 to 4.80), suggesting a higher microbial activity in the media containing sorbet [49]. This decrease in pH was slightly more pronounced in the media containing the elderberry-enriched sorbets.

Table 6. Evolution of the pH in the growth medium inoculated with probiotic bacteria without the addition of sorbet (negative control) or with the addition of blueberry sorbet (C, P, E-5, and E-50) [1].

Time (h)	pH of Growth Media				
	Negative Control	Sorbet C	Sorbet P	Sorbet E-5	Sorbet E-50
0	6.24 ± 0.01 [a]	6.12 ± 0.01 [b]	6.20 ± 0.01 [a,b]	6.20 ± 0.01 [a,b]	6.15 ± 0.01 [a,b]
3	6.24 ± 0.01 [a]	6.17 ± 0.03 [a,b]	6.20 ± 0.01 [a,b]	6.15 ± 0.01 [b]	6.20 ± 0.01 [a,b]
8	5.89 ± 0.02 [a]	5.83 ± 0.01 [a,b]	5.85 ± 0.01 [a,b]	6.87 ± 0.03 [a,b]	5.81 ± 0.01 [b]
24	5.22 ± 0.02 [a]	5.13 ± 0.01 [a,b]	5.09 ± 0.01 [a,b]	5.17 ± 0.01 [a,b]	5.04 ± 0.01 [b]
48	4.80 ± 0.01 [a]	4.59 ± 0.01 [a,b]	4.61 ± 0.01 [a,b]	4.62 ± 0.02 [a,b]	4.57 ± 0.01 [b]

[1] Results are expressed as mean values ± standard deviation. Analyses were made in triplicate. Means followed by the same letter in the same line are not significantly different ($p < 0.05$).

Table 7. Population count of probiotic bacteria (log CFU/mL) in MRS growth medium without (negative control) or with the addition of blueberry sorbet (C, P, E-5, and E-50) [1].

Time (h)	Population of Microorganisms (log CFU/mL)				
	Negative Control	Sorbet C	Sorbet P	Sorbet E-5	Sorbet E-50
0	6.22 ± 0.01 [a]	6.55 ± 0.04 [a]	6.47 ± 0.02 [a]	6.40 ± 0.01 [a]	6.47 ± 0.01 [a]
8	8.13 ± 0.02 [a]	7.76 ± 0.04 [a]	7.77 ± 0.05 [a]	7.69 ± 0.04 [a]	7.61 ± 0.03 [a]
24	8.94 ± 0.04 [a]	9.12 ± 0.03 [a]	8.96 ± 0.04 [a]	9.00 ± 0.11 [a]	9.11 ± 0.01 [a]

[1] Results are expressed as mean values ± standard deviation. Means followed by the same letter in the same line are not significantly different ($p < 0.05$).

The decrease in pH is in accordance with the results obtained in the counting of microorganisms (Table 7). After 24 h of incubation the population of probiotic bacteria expressed in log CFU/mL is higher in the media incubated in the presence of blueberry sorbet.

4. Conclusions

In collaboration with industry, it was possible to improve a blueberry sorbet formulation using natural ingredients recovered from elderberry pomace. The sorbets formulated with elderberry pomace, especially sorbet E-50, showed higher contents of phenolic compounds and anthocyanins and higher $ABTS^{\bullet+}$-scavenging activity than the control. The use of powdered pomace demonstrated a tendency to increase the content of antioxidant compounds with the advantage of not requiring the use of any solvent and additional processing steps. The addition of the more concentrated elderberry pomace extract resulted in a lower value of the color parameter L^*, which is associated with a darker hue of sorbet E-50. Other physical and the sensory characteristics of the final product were not substantially affected by the addition of pomace in the formulation.

The preliminary study of the prebiotic potential of the blueberry sorbets, especially E-50 sorbet, indicated a possible tendency to stimulate probiotic bacteria that should be explored in the future in the development of improved formulations.

Elderberry pomace, which is an undervalued by-product, can be used as source of natural ingredients with antioxidant and color properties.

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