

## Edición especial 25 años del doctorado en ingeniería

### Unleashing the potential of flash vacuum expansion: an innovative approach for andean blackberry (*Rubus glaucus Benth*) processing

Desatando el potencial de la expansión instantánea al vacío: un enfoque innovador para el procesamiento de mora de los Andes (*Rubus glaucus Benth*)

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# Abstract

Andean Blackberry fruits (*Rubus glaucus* Benth) have promising market potential with notable nutritional and antioxidant properties; however, their limited 3-6day shelf-life presents considerable challenges. A flash vacuum expansion (FVE) process coupled with vacuum de-pulping was used to obtain puree from blackberry fruits. Different steam heating holding times (53, 75, 85, and 95 s) were tested at a pressure of 130 kPa. After FVE and vacuum de-pulping (5kPa), various parameters, including alcohol insoluble residues (AIR), residual activity of polyphenol oxidase (RAPPO), anthocyanins, ascorbic acid and ellagitannin retention, rheological properties, and microbial reduction, were evaluated in the purees. Optimal steam heating time of 85 seconds was selected for the FVE process, showing outstanding retention rates of 98% for cyanidin-3-O-glucoside and 88% for ascorbic acid, along with enhanced ellagitannins extraction from torus and seed blackberries into the puree. The purees displayed a gradual shear-thinning flow behavior, positively correlated with the increase in %AIR. A reduction greater than 5 Log<sub>10</sub> CFU / mL was achieved for molds, yeasts, aerobic mesophilic, and coliforms counts for all the treatments. Nectar and sweetened puree products developed from the optimal FVE processed puree, received favorable acceptance from consumers, with a high intention to purchase. The findings demonstrated that this innovative process has great potential for developing of high-quality products.

Key words: Andean Blackberry, Flash vacuum expansion, anthocyanins, ellagitannins, innovative process

# Resumen

Los frutos de mora de los Andes (*Rubus glaucus* Benth) tienen un prometedor potencial de mercado con notables propiedades nutricionales y antioxidantes; no obstante, su vida útil limitada de 3-6 días plantea desafíos importantes. Se utilizó el proceso de Expansión Instantánea al Vacío (FVE) acoplado con el despulpado al vacío para obtener puré de mora. Se evaluaron diferentes tiempos de calentamiento (53, 75, 85 y 95 s) con vapor a una presión de 130 kPa. Se evaluaron los residuos insolubles en alcohol, la actividad residual de la enzima polifenol oxidasa (RAPPO), la retención de antocianinas, el ácido ascórbico y elagitaninos, las propiedades reológicas y la reducción microbiana en los purés después del proceso FVE y el despulpado al vacío (5kPa). Se seleccionó un tiempo óptimo de calentamiento con vapor de 85s para el proceso FVE, el cual mostró un 98% de retención para cianidin-3-O-glucósido y de 88% para el ácido ascórbico, además de una mayor extracción de elagitaninos en el puré a partir del torus y de las semillas de la mora. Los purés mostraron un comportamiento de flujo pseudoplástico creciente, correlacionado con el aumento en el %AIR. Se logró una reducción de más de 5 Log<sub>10</sub> CFU / mL en los recuentos de mohos, levaduras, mesófilos aeróbicos y coliformes para todos los tratamientos. El néctar y el puré desarrollados a partir del puré óptimo procesado con FVE, tuvieron una aceptación favorable por parte de los consumidores, con una alta intención de compra. Los hallazgos sugieren que este proceso innovador tiene un gran potencial para desarrollo de productos de alta calidad.

Palabras clave: Mora de los Andes, Expansión Instantánea al vacío, antocianinas, elagitaninos, innovador





## Introduction

Andean Blackberry fruits hold significant market potential but face a crucial challenge due to their short shelf life of 3-6 days, attributed to a high respiration rate and the lack of protective peel. This vulnerability to microbial attack and rapid deterioration presents a significant hurdle. (1,2). In Colombia, blackberries are highly consumed due to their environmental endowment and diverse cultivated and wild species, allowing successful local and international marketing (3). Enhancing preservation techniques and technical capabilities would extend their shelf life, creating greater market opportunities.

Andean Blackberries are recognized as a rich source of phenolic compounds, which offer valuable health benefits to humans. These advantages are primarily attributed to their antioxidant and anti-inflammatory properties, driven by the presence of anthocyanins and ellagitannins (4,5). Notably, anthocyanins are mainly located inside the vacuole, while ellagitannins are found in the torus and seeds (6,7).

*Rubus glaucus* Benth, commonly known as Andean blackberry, is processed by local industries in major producing countries (Colombia and Ecuador) due to the high demand for blackberry-based beverages (8). Thermal processing has been reported to significantly reduce anthocyanin content in blackberries. Moreover, losses of up to 70% of ellagitannins have been attributed to the removal of torus and seeds in the presscake, as well as hot-filling during juicing processing. (8–10) The high microbial load of this fruit, influenced by production and harvesting practices, along with its high nutritional value, presents a challenge to the industry to maintain its functional properties while eliminating the microorganisms, simultaneously ensuring its quality.

Flash Vacuum Expansion (FVE) is an innovative process that involves heating plant material between 60 and 95 °C and rapidly depressurizing it in a vacuum chamber (2 to 5 kPa). The heat treatment effectively softens the fruit tissue, while the vacuum expansion creates micro-channels through the rapid self-vaporization of the fruit's water content, resulting in efficient cooling and facilitating the extraction of bioactive compounds from the cells (11). By coupling vacuum de-pulping with FVE, it becomes possible to obtain high-quality fruit puree in a single seamless step (12). Notably, FVE offers the advantage of lower equipment costs and specific energy consumption compared to conventional methods, making it highly suitable for small and medium scale agroindustries. Its compatibility with an aseptic packaging system further enhances its appeal, providing a convenient solution for modern fruit processing (13).

Recently, Arias et al. (12,13) found that FVE significantly improved the extraction of bioactive compounds, rheological behavior, and decontamination in processed purple passion fruit and goldenberry. These promising results indicate that FVE could be an intriguing alternative for berry processing. However, despite demonstrating its effectiveness in processing fruits like grapes, guava, avocados, passion fruit, purple passion fruit, and mango (11,12,14–18), the application of FVE remains relatively limited, with no studies reported for Andean blackberries. This study sought to evaluate Flash Vacuum Expansion (FVE) as an alternative approach for crafting premium Andean blackberry puree, investigating its impact on functional compounds, rheology, microbial quality, and physicochemical attributes. Additionally, sensory evaluations measured the acceptance of two resulting products.

## Materials and methods

### Vegetal material

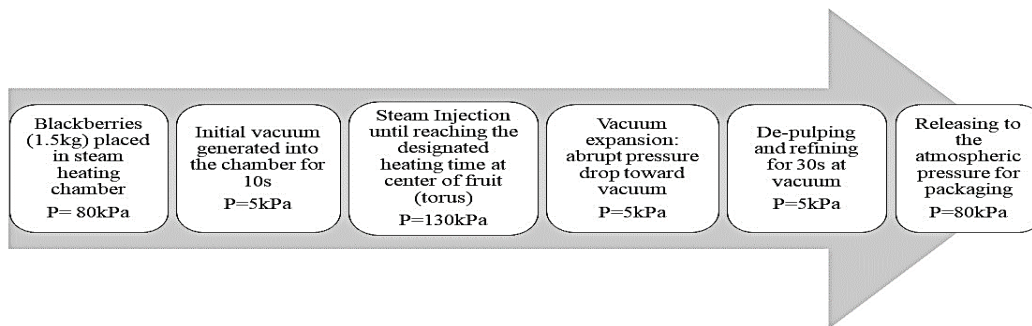
Fresh Andean blackberries (*Rubus glaucus* Benth) grow in Granada (2050 m.a.s.l), Antioquia (Colombia) were used. Specifications set by the Colombian Technical Standard NTC 4106 were fulfilled (19). Fruits with a maturation degree of 5 were employed.

### Flash-Vacuum Expansion Process (FVE)

Blackberry fruit were processed using the same pilot line of the FVE, as reported by Arias et al.(12,13). Briefly, pilot line features a stainless-steel cylindrical chamber (diameter x high = 154 x 175 mm; 6L of volume) connected to a 37.5 L vacuum expansion chamber with a rotating pulper/finisher. A large-diameter pneumatic ball valve (diameter = 15 cm) separates the chambers, operated by a fast pneumatic actuator (80% opening in 1 second). Vacuum is generated by a liquid ring pump (Robuschi RVS\_3 M-02, Parma, Italy) at a rate of 4200 m<sup>3</sup>/h, maintaining vacuum pressures of 5±1.2 kPa in the expansion chamber. Water vapor condensation minimizes gas volume drawn by the vacuum pump. Vacuum levels are monitored by a digital vacuum transducer (Sitrans P, Siemens, Germany). Inside the vacuum chamber, a rotary pulping system (until 1500 rpm) sifts fruit puree through 1 mm mesh. The setup also includes two aseptic tanks for product and co-product recovery. The entire FVE pilot line undergoes steam sterilization before each treatment, and vacuum release is facilitated through a vented sterilizing filter with an absolute air particle removal rate of 0.003 µm (Emflon® PFR Filters, Pall, Washington, NY, USA). Figure 1 presents the description of the FVE process for whole fruits. An initial vacuum was generated to enhance heat transfer during the steam heating stage, thereby reducing condensation. The steam injection holding times were set at 53, 75, 85, or 95 seconds in the heating chamber prior to the vacuum stage. Temperatures at the center of the fruit (torus) were recorded using thermocouples inserted into the fruits and connected to a data logger (DMCA-1019-2, Maycin, Medellin, Colombia). The de-pulping and refining steps under vacuum were carried out at 1300 rpm for 30 seconds using a mesh size of 1 mm. The obtained puree was immediately packaged in pre-irradiated multilayer bags (plasticized, PET/Foil/LPDE 120 microns, Smurfit Kappa®, Dublin, Ireland) using a bag-in-box semimanual filler (Sympaty ROp 320, Technibag, Villefanche-sur-Seine, France) set up inside a laminar flow hood. Technology readiness levels (TRLs) are a method for estimating the maturity of technologies. Under those operational conditions, the capacity of the pre-commercial pilot plant at TRL 7 is 50 kg/L, with a 6-hour operational period, including 2 hours for cleaning. Therefore, during a 6-hour workday, it would be possible to process a total of 300kg of blackberries. It is important to consider that the line is a pilot-scale system, so real-world validation may lead to increased flows and yields.



Figure 1. Main stages of FVE treatment to obtain blackberry purees.



Four replicates were performed for each treatment. All the tests were conducted in La Selva Research Center (Agrosavia) Rionegro (Antioquia, Colombia) at an elevation of 2100m.a.s.l, corresponding to an average atmospheric pressure of approximately 80 kPa. Raw puree was obtained by simply de-pulping the fresh fruit and sieving it at 1300 rpm for 30 seconds using a 1 mm mesh size.

### Physicochemical Characterization

The pH, titratable acidity, and soluble solids were determined by AOAC 2005 standard methods (981.12, 942.15, and 932.12, respectively). Color was determined from CIELAB space (HunterLab ColorFlex EZ spectrophotometer, primary illuminant D65, observation angle 10°).

Alcohol insoluble residues (AIR) was measured following protocol described by Haminiuk et al. (20). Suspended Insoluble Solids (SIS) was evaluated as the percentage by weight of the residue after centrifugation of the raw and processed puree (10 g at 3300 g x 15min) (21). Residual activity of polyphenol oxidase (RA PPO) was determined by comparing the enzymatic activity of the processed puree to that of the raw puree, following the protocol reported by Cervantes et al (22) for blackberries with slight modifications. Enzyme extraction was conducted in the same way, but PPO activity was measured using the protocol described by Gonzalez et al. (23).

### Determination of Anthocyanins and Ellagitannins

Extraction was performed according to García-Villalba et al. (24). Puree (1.5 g) was added to 5 mL of the acidic water (1.35% v/v HCl), stirred for 30 min at room temperature, and then centrifuged for 10 min (3000 rpm, 20 °C). The supernatant was collected and 4 mL of the methanol/DMSO/acidic water mixture (40:40:20) were added, then it was centrifuged for 10 min (3000 rpm, 20 °C). Finally, the supernatants were collected and brought up to 10 mL with methanol, and the liquid obtained was filtered with a 0.45-µm PVDF membrane (Syringe Filter, Quality Laboratory Supplies, Miami, FL, USA). Anthocyanin and ellagitannin content in different puree was determined at 515 nm (Cyanidin 3-O-glucoside) and at 254nm (Ellagic acid) by HPLC (Prominence 20, Shimadzu, Kyoto, Japan) equipped with a PDA (SPD-M20A, Shimadzu, Kyoto, Japan) detector. The mobile phase used was 2% formic acid and acetonitrile: water:formic acid (80:18:2). The flow rate was 0.4 mL/min, and the injection volume was 2 µL. A C18 100-

Å 5- $\mu\text{m}$  column (250  $\times$  4.6 mm, Phenomenex Luna, Torrance, CA, USA) was used, and the column temperature was 30°C (24). Anthocyanins and ellagitannins were quantified using calibration curves of cyanidin-3-glucoside (44689-5MG, Sigma, Merck, Darmstadt, Germany) and ellagic acid (14668-50MG, Sigma, Merck, Darmstadt, Germany) with correlation coefficients of 0.997 and 0.998 respectively. Lambertianin C and Sanguin H6 were expressed in terms of ellagic acid content.

### Determination of Ascorbic Acid

The extraction and identification of ascorbic acid was carried out according to the methodology described by Lee et al. (25) with some modifications, as described below. Approximately 3g of puree were weighed, 20 mL of a  $\text{KH}_2\text{PO}_4$  solution (0.02 M, pH: 3.06 adjusted with 85% Ortho-Phosphoric Acid) were added, the mixture was stirred at room temperature (20°C), for 1 min at a speed of 3000 rpm with a vortex (Analog vortex mixer, VWR; Avantor delivered by VWR, United States). Subsequently, it was centrifuged at 4°C for 15 minutes at 3000 rpm and the supernatant was filtered using a 0.45 $\mu\text{m}$  PVDF syringe filter. Identification and quantification were performed by HPLC (Prominence UFLC 20A, Shimadzu, Kyoto, Japan), coupled to a Prominence SPD-M20A diode array detector, Luna® C18 column (2) 100 Å (250 mm\* 4.6 mm ID\* 5,0 $\mu\text{m}$ ), mobile phase the same extraction solution. The analysis conditions were mobile phase flow rate 1.0 mL/min, temperature 35°C, injection volume 20 $\mu\text{L}$ , absorption wavelength at 244nm, and isocratic mode. the concentration of ascorbic acid in the purees was determined by the external standard method, using ascorbic acid standard curve (Sigma Aldrich 47863) (0.1 – 50 $\mu\text{g}/\text{mL}$ ),  $R^2 = 0.999$ , retention time was 4.37 min. The results were expressed as mg ascorbic acid/100 g puree on fresh weight, FW.

### Microbiological Analyses

Culture media were used by the deep sowing method on Petri dishes. Puree (10 g) was mixed with 90 mL of sterile peptone water (0.1% w/v). A tenfold dilution series was prepared in sterile peptone water for plating. The following culture media and conditions were used to enumerate the microbial cells: 1. Mesophilic aerobic bacteria count (DEV nutrient agar, Merck), incubated at 37 °C for 2 days; 2. Mould and yeast (Sabouraud 4% dextrose agar, Merck), incubated at 25 °C for 5 days; 3. Faecal and total coliforms (Chromocult medium agar, Merck), incubated at 37 °C for 2 days. All analyses were performed in triplicate. The results are reported as log CFU/g of the sample (fresh weight, FW).

### Rheological properties

Flow curves were used to evaluate the effect of FVE on the rheological properties of the fruit puree. The rheological measurements were performed using an Anton rheometer (Paar brand MCR 92, Graz, Austria) equipped with Rheocompass® software (v.1.20, Anton Paar, Graz, Austria) and a C-CC27 concentric cylinder geometry (26.6 mm diameter). Flow curves were obtained following a similar method as described by Varga-Tóth et al. (26). Approximately 20 mL of each sample was taken, and the shear stress ( $\tau$ ) was measured as a function of shear rate in three phases at 25°C: an ascending curve (0.01–200 $\text{s}^{-1}$  for 60s), holding time (200s–1 for 120s), and a descending curve (200–0.01 $\text{s}^{-1}$  for 60s) (12). Data from the descending curve were fitted to Ostwald da Waele



model (best fit) and the threshold stress ( $\sigma_0$ ), the consistency index (K), and flow behavior (n) for each FVE process was estimated. This model achieved the best fit with the highest determination coefficient ( $R^2$ ) and the lowest values for chi-square ( $\chi^2$ ) and the sum of squared residuals (SSR) (25). The apparent viscosity of the purees was calculated at a shear rate of  $50\text{s}^{-1}$ .

### Sensory Analysis: Nectar and Sweetened puree

Prior to conducting the consumer test, blackberry nectar (3:2 puree:water rate, 11°brix) and sweetened puree (7% added sugar) were prepared using FVE-processed puree (85s heating time). For the sensory analysis, 118 regular consumers of fruit processing products were recruited comprising 58% female and 42% male participants, with ages ranging from 14 to 59. Acceptance tests were performed to evaluate attributes such as aroma, color, flavor, and overall acceptance using a 5-point structured hedonic scale (1=extremely dislike, 5=extremely like). The 5-scale just about right (JAR) test was employed to measure attribute intensity for sweetness, sourness, and consistency of purees (1=very strong, 3=JAR, 5=very weak). Finally, the intent of purchase was evaluated, including the possible intended use, particularly for sweetened puree.

### Statistical Analysis

FVE processes were conducted using a completely randomized design with 4 replicates. All quality analyses were performed in triplicate, and data are expressed as the mean  $\pm$  standard deviation. Before ANOVA, normality and homoscedasticity were analyzed (Shapiro-Wilk normality and Levene homoscedasticity tests, respectively). Then, ANOVA and Tukey's ( $p < 0.05$ ) test were performed to assess significant differences between treatments. Data analysis was carried out using the statistical software MINITAB® 18.1. Sensory analysis was done by a Liking data analysis and JAR (just about right test) (XLSTAT 2023.1.4, Lumivero (2023). XLSTAT statistical and data analysis solution. New York, USA. <https://www.xlstat.com/es>)

## Results and discussion

Figure 2 shows the different steps of the process considering surrounding pressure and temperature at the center of the fruit. In stage I of FVE process, an initial vacuum (5kPa) is generated in the heating chamber to enhance the heat diffusivity during the high-pressure steam heating (130kPa) that takes place in stage II. Heating chamber's holding times at 130 kPa of 53, 75, 85 and 95 s correspond to recorded center fruit of approximately 60, 70, 80 and 90°C, respectively, as measured by thin thermocouples. In stage III when the steam-heating step is performed, the fruit undergoes a rapid pressure drop of up to 5kPa, resulting in bursting. Subsequently, the fruit rapidly cools, reaching temperatures around  $35 \pm 5^\circ\text{C}$  at its core. During stage IV, the fruit is depulped and finished under vacuum pressure (5kPa). Finally, in stage V, the pressure is released to proceed with aseptic packaging.

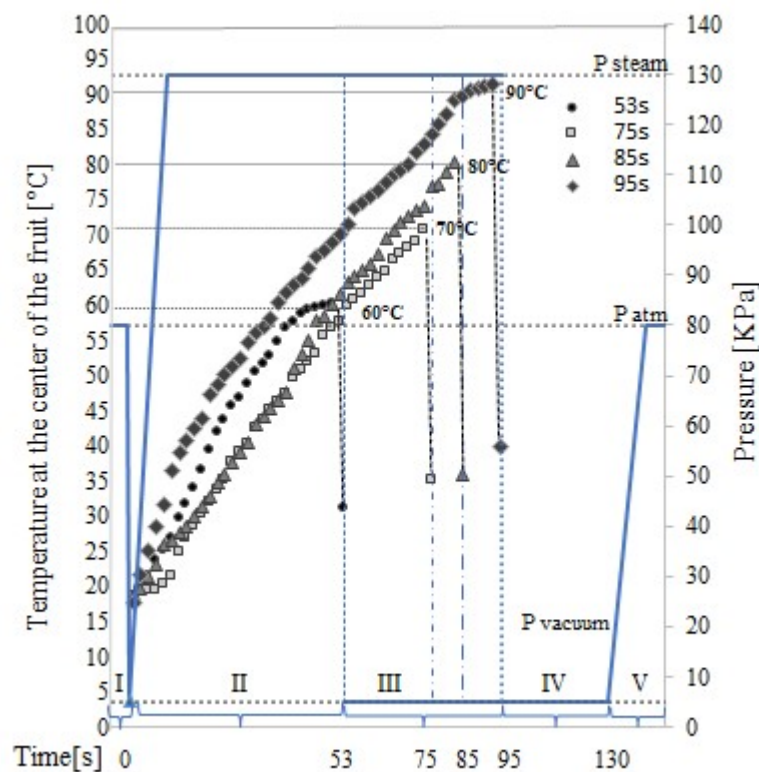


Figure 2. Temperature profile at the center of the fruit (torus) and pressure in the surrounding area during the Flash Vacuum Expansion process. P: pressure, atm: atmospheric

### Physicochemical characterization of puree

In table 1, no statistically significant differences were found between the assessed heating levels for both pH and soluble solids ( $p > 0.05$ ). Nevertheless, a notable decrease in titratable acidity, along with a marginal increment in pH, was observed in the blackberry purees subjected to FVE treatment ( $p < 0.05$ ) compared to raw puree. This phenomenon could be attributed to the incorporation of fruit components, such as the torus or fleshy receptacle, which undergo partial disintegration during the process. Similar observations have been reported by Brat et al.(14) y Arias et al. (12).

The exposure time of the fruit to saturated steam resulted in a significant increase ( $p < 0.05$ ) in both Suspended Insoluble Solids (SIS) and Alcohol-insoluble residues (AIR) compared to raw puree, reinforcing the effective disintegration of plant tissues. However, beyond 75 s of heating, no statistically significant differences were observed ( $p > 0.05$ ) in the SIS content. Furthermore, the AIR displayed an increase across all treatments ( $p < 0.05$ ), particularly at 85s of heating, where it achieved a substantial 1.6-fold increment compared to the raw puree. Similar results have been reported in mango, guava, and passion fruit (14) and purple passion fruit (12) after FVE treatment. This is the first reported study for blackberry treated with this technology.





The residual activity percentage (%RA) of polyphenol oxidase (PPO) in blackberry purees decreased to 16% after FVE treatment ( $p < 0.05$ ) compared to the raw puree, as the saturated steam heating time increased (Table 1). This findings aligns with the results reported by Noreña et al. (27) who observed a decrease of up to 36% in the residual activity of PPO after exposing blackberries to 10min of steam heating at 100°C. The FVE treatment utilizes saturated steam at 130kPa (121°C) which could explain the lower %RA of polyphenol oxidase observed after only 95s. The remain activity may be attribute to the presence of isoenzymes, with a heat-labile fraction that can be completely inactivated and a heat-resistant fraction that cannot be fully inactivated. (27)

Although no apparent variation in the color coordinates of the fruit puree was observed among the FVE treatments, a significant decrease was evident compared to the raw puree ( $p < 0.05$ ). The presence of soluble and insoluble pectin, cellulose, hemicellulose, lignin, and other cell wall compounds in both SIS and AIR may impede light dispersion, leading to lower values in the color coordinates. A similar behavior was observed in purple passion fruit (12) and goldenberry (28) purees after FVE treatment. All treatments showed a  $\Delta E$  greater than 3, indicating a noticeable visual difference (29) compared to the raw puree.

Table 1. Effect of steam heating holding time of FVE process on the physicochemical variables of blackberry purees.

| Parameters                          | Raw Puree                 | Steam Heating Time (seconds) |                            |                           |                            |
|-------------------------------------|---------------------------|------------------------------|----------------------------|---------------------------|----------------------------|
|                                     |                           | 53                           | 75                         | 85                        | 95                         |
| pH                                  | 2.80 ± 0.03 <sup>b</sup>  | 2.98 ± 0.04 <sup>a</sup>     | 3.00 ± 0.02 <sup>a</sup>   | 2.98 ± 0.01 <sup>a</sup>  | 2.98 ± 0.01 <sup>a</sup>   |
| Acidity (%malic acid)               | 2.29 ± 0.08 <sup>a</sup>  | 1.99 ± 0.05 <sup>b</sup>     | 1.73 ± 0.02 <sup>c</sup>   | 1.77 ± 0.01 <sup>c</sup>  | 1.97 ± 0.01 <sup>b</sup>   |
| Soluble solids (gxL <sup>-1</sup> ) | 7.84 ± 0.27 <sup>a</sup>  | 7.52 ± 0.42 <sup>a</sup>     | 7.82 ± 0.28 <sup>a</sup>   | 7.60 ± 0.36 <sup>a</sup>  | 7.66 ± 0.15 <sup>a</sup>   |
| SIS <sup>1</sup> (g/100 g FW)       | 17.98 ± 0.70 <sup>c</sup> | 31.95 ± 0.72 <sup>b</sup>    | 36.72 ± 0.62 <sup>a</sup>  | 37.66 ± 0.04 <sup>a</sup> | 38.10 ± 0.61 <sup>a</sup>  |
| AIR <sup>2</sup> (g/100 g FW)       | 0.95 ± 0.02 <sup>d</sup>  | 1.23 ± 0.01 <sup>c</sup>     | 1.28 ± 0.07 <sup>b</sup>   | 1.48 ± 0.02 <sup>a</sup>  | 1.46 ± 0.01 <sup>a</sup>   |
| RA PPO <sup>3</sup> (%)             |                           | 53.10 ± 1.28 <sup>a</sup>    | 45.74 ± 1.07 <sup>b</sup>  | 19.87 ± 0.20 <sup>c</sup> | 16.06 ± 0.66 <sup>d</sup>  |
| L                                   | 16.46 ± 0.33 <sup>a</sup> | 14.76 ± 0.12 <sup>b</sup>    | 14.66 ± 0.10 <sup>bc</sup> | 14.26 ± 0.09 <sup>c</sup> | 14.51 ± 0.14 <sup>bc</sup> |
| a*                                  | 37.51 ± 0.17 <sup>a</sup> | 34.80 ± 0.04 <sup>b</sup>    | 34.26 ± 0.03 <sup>c</sup>  | 33.65 ± 0.10 <sup>d</sup> | 34.09 ± 0.02 <sup>c</sup>  |
| b*                                  | 19.13 ± 0.17 <sup>a</sup> | 15.15 ± 0.13 <sup>b</sup>    | 14.30 ± 0.03 <sup>c</sup>  | 14.18 ± 0.04 <sup>c</sup> | 14.32 ± 0.05 <sup>c</sup>  |
| $\Delta E$                          |                           | 5.10 ± 0.19 <sup>b</sup>     | 6.09 ± 0.27 <sup>a</sup>   | 6.65 ± 0.35 <sup>a</sup>  | 6.21 ± 0.29 <sup>a</sup>   |

<sup>1</sup> Suspended insoluble solid. <sup>2</sup> Alcohol-insoluble residues. <sup>3</sup> Residual Activity of Polyphenol-oxidase enzymes. Values are presented as the mean ± standard error, n = 3, different letters in each row indicate that there is a statistically significant difference ( $p < 0.05$ ), FW: fresh weight.

The main anthocyanin in blackberries (cyanidin-3-O-glucoside), along with the two ellagitannins (lambertianin C and sanguinin H6), ellagic acid and ascorbic acid were quantified and expressed as retention percentages relative to the initial contents in the raw puree (Figure 3). The results indicate that there was no degradation of cyanidin-3-O-glucoside at the evaluated heating times ( $p > 0.05$ ), with an average value of 514.25 mg/100g dry weight (dw). Thermal stability of anthocyanins was demonstrated by

Brownmiller et al. (30) after steam blanching of entire blueberries. The observed slight increase (Fig 3) could be attributed to the heat-induced disruption of the cell wall and the vacuum expansion, leading to the release of anthocyanins from the vacuoles. Furthermore, Gancel et al. (8) discovered that cyanidin malonyl-glucoside exhibited less stability during blanching in blackberries (41% loss), and it might be converted into cyanidin-3-O-glucoside, thereby mitigating adverse effects on its concentration. Lower cyanidin values were reported for *Rubus Glaucus* Benth species in Colombia (264.4mg/100g dw) (3) and Ecuador (380 mg/100g dw) (5) compared to our study.

After FVE treatment, both ellagitannins exhibited a significant increase ( $p < 0.05$ ) the heating time extended up to 85s, possibly influenced by the bursting of the torus and tissues around the seeds during the abrupt expansion of the fruit after the vacuum step. Sójka et al (31) reported comparable results, observing an increase in the content of these ellagitannins up to 80°C during the first 2 hours in aqueous solutions with a pH similar to that of blackberries. However, at 95s of steam heating, a subsequent decrease was observed ( $p < 0.05$ ), likely resulting from their partial hydrolysis into insoluble ellagic acid during the heat treatment (8), particularly in lambertianin C. The higher thermal stability of sanguin H6 compared to lambertianin C, can be attributed to their distinct molecular structures, with sanguin H6 being a dimer and lambertianin C a trimer (8). The variation in ellagic acid content with heating time displayed unpredictable patterns, possibly attributed to its formation as a by-product during ellagitannin degradation. The higher content of ellagic acid and ellagitannins at 85s may be attributed to an increased presence of cell wall compounds under these conditions (%AIR), leading to their polymerization and eventual covalent attachment to cell wall fragments, which could prevent hydrolysis (10). In summary, the FVE process facilitates a higher extraction and incorporation of ellagitannins into the puree, thereby enhancing its functional value.

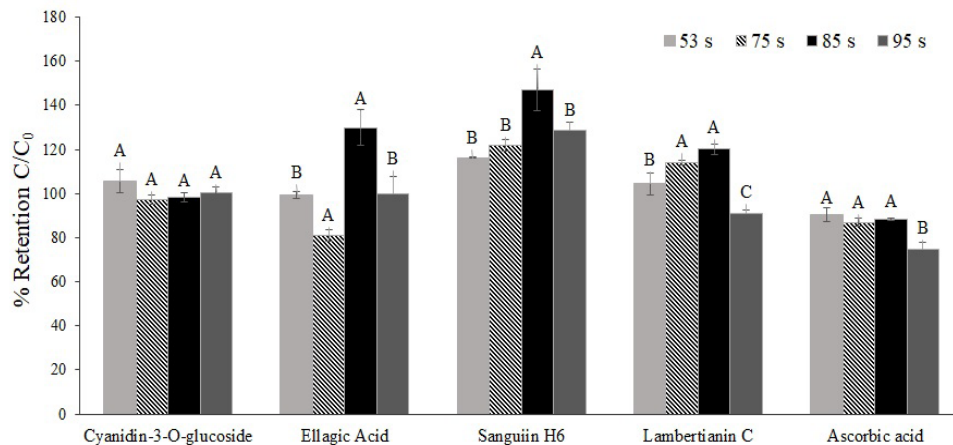


Figure 3. Retention of anthocyanins, ellagitannins, and ascorbic acid in processed blackberries at different heating times during the Flash Vacuum Expansion process compared to untreated blackberry puree. C: final concentration of compound after processing,  $C_0$ : initial concentration of compound in raw puree. Mean values with common letters shown above the bars are not statistically different among heating time at a P value of  $< 0.05$ .



Figure 3 shows the significant impact ( $p < 0.05$ ) of heating time on ascorbic acid content compared to the raw puree. The ascorbic acid content in the raw puree, approximately  $17.06 \text{ mg} \cdot 100 \text{ g}^{-1}$  fresh weight, was comparable to that reported by Garzón et al. (32) in *Rubus Glaucus Benth* from Bogotá, Colombia. The FVE process exhibited a significant impact among the treatments ( $p < 0.05$ ) after 95 seconds, resulting in a retention of 74.6% of ascorbic acid (approximately  $13.28 \text{ mg} \cdot 100 \text{ g}^{-1}$  fresh weight). The ascorbic acid retention in FVE blackberry purees with holding times ranging from 53s to 85s (approximately 91-88%) was found to be similar to that reported for goldenberry purees processed by FVE for 40s (approximately 88%)(28). These results can be compared to those found in blueberries juice treated at 400 and 600MPa for 5-15min in High Pressure Processing (92%) (33). Overall, the favorable retention of anthocyanins and ascorbic acid in blackberry purees treated by FVE could be attributed to the short thermal exposure times and the instantaneous cooling of the final product resulting from depressurization during the expansion stage.

### Rheological properties of puree

Table 2 clearly demonstrates the noticeable impact of vacuum expansion on the rheological behavior of the purees under different heating times. All fruit purees showed a shear-thinning flow behavior with "n" values below 1. A significant increase ( $p < 0.05$ ) in all rheological parameters is observed for FVE treatments compared to the raw puree, particularly for K and apparent viscosity ( $\eta$ ) at 85s. This behavior is closely related to the increase in AIR and SIS as previously reported in the puree, strongly suggesting the incorporation of soluble pectin. This results in higher viscosity, enhanced homogeneity, and improved stability of the puree after processing and in subsequent product developments. Similar results were reported for passion fruit, guava, mango (14), and purple passion fruit after FVE treatment (12), with the latter attributing a "smoothie" characteristic to the obtained purees. In summary, the FVE process facilitated the release of phenolic compounds and potential incorporation of soluble pectin in the puree, leading to enhanced homogeneity through the formation of hydrocolloidal suspensions. This was evident from the observed increase in the analyzed rheological parameters.

Table 2. Effect of steam heating holding time of FVE process on the rheological parameters of blackberry purees.

| Parameters  | Raw Puree           | Steam Heating Time (seconds) |                     |                     |                     |
|---|---------------------|------------------------------|---------------------|---------------------|---------------------|
|   |                     | 53                           | 75                  | 85                  | 95                  |
| $\sigma_0$ (kPa)                                  | $0.295 \pm 0.004^e$ | $1.222 \pm 0.019^d$          | $1.494 \pm 0.060^c$ | $1.893 \pm 0.069^a$ | $1.659 \pm 0.038^b$ |
| n   | $0.327 \pm 0.004^b$ | $0.348 \pm 0.007^a$          | $0.354 \pm 0.002^a$ | $0.358 \pm 0.004^a$ | $0.354 \pm 0.004^a$ |
| K (mPa.s <sup>n</sup> )                           | $0.082 \pm 0.007^e$ | $2.557 \pm 0.244^d$          | $2.986 \pm 0.015^c$ | $4.145 \pm 0.176^a$ | $3.619 \pm 0.140^b$ |
| SSR   | $0.059 \pm 0.005$   | $0.075 \pm 0.003$            | $0.073 \pm 0.001$   | $0.072 \pm 0.005$   | $0.064 \pm 0.002$   |
| $\chi^2$  | $0.096 \pm 0.016$   | $0.196 \pm 0.012$            | $0.152 \pm 0.004$   | $0.101 \pm 0.010$   | $0.079 \pm 0.003$   |
| $R^2$   | $0.981 \pm 0.008$   | $0.991 \pm 0.002$            | $0.992 \pm 0.001$   | $0.993 \pm 0.002$   | $0.995 \pm 0.001$   |
| $\eta$ at $\sigma^{50 \text{ s}^{-1}}$<br>(mPa.s) | $18.53 \pm 0.881^e$ | $163.3 \pm 6.124^d$          | $232.5 \pm 5.018^c$ | $336.2 \pm 4.646^a$ | $266.3 \pm 4.525^b$ |

Mean  $\pm$  standard error, n = 3, different letters in each row indicate that there is a statistically significant difference ( $p < 0.05$ ). Threshold stress ( $\sigma_0$ ); average flow behavior (n); average consistency index (K), complex viscosity ( $\eta$ ) at shear rate of  $50 \text{ s}^{-1}$  ( $\eta^{50 \text{ s}^{-1}}$ ).

## Microbiological quality of puree

Table 3 shows the plate microbial content of the raw puree, steam heated (stage 1), and FVE processed puree at different heating times. The raw purees of blackberry fruit presented a count of mesophilic aerobic microorganisms, molds and yeasts, total and fecal coliforms of 3.5, 7.7, and 0 log CFU/g, respectively. After the FVE process, the microbial counts were always below the detection limit for all the microorganisms evaluated in all the treatments.

Table 3. Viability of aerobic mesophilic bacteria, molds and yeasts, and fecal and total coliforms in blackberry purees.

| Treatment               | Plate microbial content [CFU/g] |                               |                           |
|-------------------------|---------------------------------|-------------------------------|---------------------------|
|                         | Aerobic mesophilic bacteria     | Molds and yeasts              | Fecal and total coliforms |
| Initial count raw puree | $3.48 \times 10^3 (\pm 15.3)$   | $7.67 \times 10^6 (\pm 10.5)$ | < 10 ( $\pm 0$ )          |
| Steam Heating           |                                 |                               |                           |
| 53s                     | $2.57 \times 10^1 (\pm 5.13)$   | $2.67 \times 10^3 (\pm 5.77)$ | < 10 ( $\pm 0$ )          |
| 75s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| 85s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| 95s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| Flash Vacuum Expansion  |                                 |                               |                           |
| 53s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| 75s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| 85s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |
| 95s                     | < 10 ( $\pm 0$ )                | < 10 ( $\pm 0$ )              | < 10 ( $\pm 0$ )          |

Mean  $\pm$  standard error, n = 3

One of the main issues affecting the shelf life of fresh blackberries is their high susceptibility to mold and yeast attacks, which is reflected in elevated raw puree counts (Table 3) (34). In this study, FVE significantly reduced molds and yeasts in the purees by 6 log CFU / g, eliminating aerobic mesophilic bacteria. These findings suggest FVE as a commercially viable method, achieving similar commercial sterility to high temperature-short time (HTST) pasteurization (6.3 log CFU/mL; 72°C, 15s) (35). These results are in agreement with other authors for FVE fruit process (12,28). Furthermore, steam heating at 53s was insufficient to achieve full microbial reduction, suggesting that a thermomechanical effect, generated by the rapid pressure change on the cell membrane, could lead to irreversible changes such as cell denaturation, protein denaturation, and membrane rupture. (36).

## Sensory evaluation of developed products

Both the blackberry nectar and sweetened puree developed from the processed blackberry puree by FVE showed good acceptability among consumers, with an average score of  $4.01 \pm 0.16$  (4=Like) for the attributes of aroma, color, flavor, and overall acceptance. Only two recent studies have demonstrated the sensory acceptance of fruit puree processed by FVE (12,37). This is the first reported consumer test for products developed from fruit puree processed by FVE.



The Just About Right (JAR) analysis for sweetness, sourness and consistency in both products were presented in Table 4. In general, an attribute is considered to reach its Just About Right (JAR) level when it scores  $\geq 75\%$ . Consequently, when an attribute deviates by more than 20% above or below the JAR level on either side, it often indicates that improvement is needed (38). The results revealed that to achieve JAR score, it is necessary to reduce the sourness of the sweetened puree by increasing its sweetness, which, consequently, also improves its consistency. To achieve JAR for the nectar, lower consistency by reducing pulp content, resulting in decreased sourness, but require increased sweetness.

**Table 4. Just About Right (JAR) consideration of blackberry nectar and sweetened puree**

| Attributes  | Intensity [%] |     |            | Consideration |
|-------------|---------------|-----|------------|---------------|
|             | Too weak      | JAR | Too strong |               |
| Nectar      |               |     |            |               |
| Sweetness   | 23 (4.19)     | 59  | 18         | Increase      |
| Sourness    | 1             | 52  | 47 (1.76)  | Decrease      |
| Consistency | 13            | 66  | 21 (1.65)  | Decrease      |
| Sweetened   |               |     |            |               |
| Puree       |               |     |            |               |
| Sweetness   | 52 (4.26)     | 45  | 3          | Increase      |
| Sourness    | 14            | 56  | 30 (1.8)   | Decrease      |
| Consistency | 25 (4.08)     | 66  | 9          | Increase      |

Just-about-right (JAR) scale was scored on a 5-point scale where 1 to 2 = Too strong, 3 = JAR, and 4 to 5 = Too weak. The JAR results indicate the percentage of consumers that selected these options; the number in the parentheses is the mean decrease calculated when the percentage of citations exceeded 20%.

Consumer purchase intent analysis revealed that 90% would buy the sweetened puree mainly for preparing pulpy fruit juices and for making preserves or desserts. As for the nectar, 70% of consumers would choose blackberry nectar despite potentially having a higher cost (approximately 10%) compared to other commercial juices with a similar fruit percentage.

## Conclusions

This study highlights the potential of flash vacuum expansion (FVE) treatment, in conjunction with vacuum de-pulping, across different steam heating durations (53, 75, 85 and 95s). The approach effectively eliminates microbial load in blackberry purees while preserving biologically active compounds and enhancing their rheological properties. Based on the results obtained, an optimal steam heating time of 85 seconds was selected for the FVE process, as it exhibited exceptional retention rates of 98% for cyanidin-3-O-glucoside and 88% for ascorbic acid, along with enhanced ellagitannins extraction from torus and seed blackberries into the puree. Furthermore, the increase in complex viscosity at this condition, which was correlated with the rise in %AIR, enabled the development of both nectar and sweetened puree products that received favorable

acceptance from consumers, with a high intention to purchase. The findings suggest that this innovative approach holds considerable promise for enhancing fruit processing methods and product quality.

## Patents

The authors declare that the FVE equipment used in the present work has been protect by the patent NC2021/0016741 (Resolución N° 41907, Dispositivo para la producción de purés, nectares o extractos de material vegetal. Superintendencia de industria y comercio Colombia).

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