

Article

Integrated Flash Vacuum-Expansion and Cryostructuring for Resource-Efficient Tamarillo Biomass Processing

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Abstract

Tamarillo (*Solanum betaceum*) processing is characterized by early biomass exclusion and thermal stabilization, which may limit in-process retention of phytochemicals. This study evaluated an integrated sequence combining Flash Vacuum Expansion (FVE) under different processing conditions with whey protein-based cryostructuring as a strategy to enhance the redistribution and structural immobilization of tamarillo bioactives. FVE promoted migration of phenolics and pigments prior to mechanical fractionation. Selected FVE-treated puree was incorporated into a whey protein matrix and subjected to cryostructuring and freeze-drying to generate a porous stabilization scaffold. Structural characterization by scanning electron microscopy and gas adsorption confirmed the formation of an interconnected porous matrix. Cryostructuring reduced water activity to 0.17 ± 0.01 and produced high porosity (91.9%) with low bulk density ($0.109 \text{ g}\cdot\text{cm}^{-3}$). Total phenolic retention exceeded 83%, while anthocyanins showed greater sensitivity (46% retention). No statistically significant additional losses of phenolics or antioxidant activity were observed during cryostructuring relative to gelation. The integrated approach illustrates a process-level stabilization pathway in which redistributed phytochemicals are physically confined within a porous scaffold, providing a structurally differentiated alternative to conventional drying for improved in-stream resource utilization.

Keywords: flash vacuum expansion; phytochemical retention; process integration; whey protein concentrate; agro-industrial by-products; porous scaffolds



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1. Introduction

The tamarillo (*Solanum betaceum*) value chain exhibits systemic inefficiencies despite the fruit's recognized nutritional and phytochemical potential [1,2]. Although rich in phenolic compounds, carotenoids, vitamin C, and anthocyanins, its industrial utilization remains limited, with processing typically restricted to fresh consumption or basic pulp-derived products [3]. Postharvest losses are influenced not only by senescence but also by aesthetic deterioration of the peduncle and calyx and by physiological disorders such as chilling injury [3]. In addition, nearly half of the fruit biomass—primarily peel and seeds—is removed during conventional processing [4]. Since this discarded fraction contains significant phenolic concentrations and higher antioxidant activity than the mesocarp [5], early fraction exclusion diverts high-value phytochemicals from the primary processing flow, limiting in-process resource utilization in fruit-based supply chains.

Conventional stabilization relies largely on thermal unit operations such as pasteurization, blanching, and spray drying to ensure microbial safety and shelf-life extension [3,4,6]. While effective from a microbiological perspective, these treatments expose bioactives to elevated temperatures and oxidative environments that accelerate degradation kinetics [1,7]. For instance, increasing processing temperatures from 80 to 90 °C has been associated with reductions of approximately 14–22% in carotenoids [7], and spray drying at inlet temperatures of 120–160 °C may induce decomposition or polymerization of thermolabile polyphenols [8,9]. In parallel, pulp is typically separated for product development while peel and seeds are excluded due to sensory constraints [2], reinforcing a linear stabilization sequence in which phenolic-rich fractions are removed prior to preservation.

Non-thermal technologies, such as high-pressure homogenization and ultrasonication, have been investigated to enhance the extractability of bound phenolics and pigments by disrupting the matrix [4,10,11]. However, when applied as isolated steps, liberated compounds may remain vulnerable to oxidation and degradation in the absence of immediate structural stabilization. Thus, coupling biomass disruption with a stabilization step becomes relevant when the objective extends beyond extraction yield toward retention within the processing chain [2,4].

Flash Vacuum Expansion (FVE) is a controlled disruption strategy that enhances intracellular release via rapid pressure drop and structural disintegration [12]. Plant material is heated with steam at atmospheric pressure (≈ 101.3 kPa), typically between 60 and 95 °C, followed by an instantaneous transfer into a vacuum chamber operating at 2–5 kPa, which induces autoevaporation of intrinsic water and transient microchannel formation [12,13]. By intensifying mass transfer, FVE can promote migration of phenolics and pigments from peel- and seed-associated tissues into the main product stream prior to mechanical separation steps that typically exclude peel- and seed-derived fractions. Nevertheless, once redistributed, these compounds enter a higher-mobility state, which may increase their susceptibility to oxidative and hydrolytic degradation during subsequent handling.

This creates a critical processing gap: although FVE enhances the release and redistribution of intracellular bioactive compounds, it does not inherently provide a mechanism for their immediate stabilization. Consequently, the disruption of cellular compartmentalization increases the exposure of phenolic compounds to reactive species and enhances molecular mobility, potentially accelerating oxidative and hydrolytic degradation in the absence of structural confinement [14,15].

Cryostructuring (ice-templating) provides a low-temperature architectural approach for immobilizing solutes within porous biopolymeric matrices [16]. During controlled freezing, solvent crystallization induces cryoconcentration, confining solutes and polymer precursors into an unfrozen liquid microphase (UFLMP) where network consolidation occurs; subsequent sublimation preserves this organization, yielding supermacroporous

structures characterized by high void fraction [16–18]. In analogous systems, compact pore walls and reduced water activity after lyophilization have been associated with decreased molecular mobility and slower degradation kinetics, effectively shielding thermolabile antioxidants from oxidative and environmental stress [18–20]. In this context, cryostructuring constitutes a stabilization format based on architectural confinement rather than rapid moisture removal alone.

Protein-based matrices, particularly whey protein systems, offer a functional platform for implementing such architectures [21]. Whey protein concentrate, an abundant by-product of cheese manufacturing [17], can form mechanically stable porous networks upon cryostructuring [21]. In the present system, it functions as the structural scaffold that physically confines the redistributed tamarillo bioactives within the porous matrix. Such networks have been reported to enable solute entrapment within interconnected channels while maintaining structural integrity [16,21].

Accordingly, the present study evaluates an integrated processing sequence that combines mild FVE as a controlled biomass disruption step with whey protein-based cryostructuring as an architectural stabilization platform. The objective is to examine how this coupled approach influences phytochemical redistribution and retention within the stabilized matrix, and to assess the resulting cryostructured format as a structurally differentiated stabilization pathway relative to conventional atomized powders in fruit-based systems.

2. Materials and Methods

2.1. Preparation of Raw Materials

The raw material was obtained from Coscomatepec de Bravo, Veracruz, Mexico, located at an altitude of 1708 m above sea level. The selected fruits were ripe and suitable for consumption. They were washed with a 120 ppm sodium hypochlorite solution and stored at room temperature until further processing.

2.2. Flash Vacuum-Expansion Treatment

For each treatment, approximately 1 kg of intact fruits was placed in the heating chamber and subjected to the following processing times: 5 (FVE_{5min}), 10 (FVE_{10min}), and 15 (FVE_{15min}) minutes at atmospheric pressure (101.3 kPa). The internal temperature of tamarillo fruit in the heating chamber was measured using Z-type thermocouples, coupled to a database system (model 92000-05, Cole-Parmer, Vernon Hills, IL, USA). The internal temperatures recorded for each treatment were 65, 77, and 91 °C for 5, 10, and 15 min, respectively. Once processing was complete, the fruit was immediately transferred to the vacuum chamber (3 kPa), where it expanded. The resulting puree was recovered, and the seed and peel residues were separated using a food-grade strainer. A fourth treatment was performed using a food-grade homogenizer at room temperature, and was used as a control. The purees were stored at −5 °C in amber-colored bottles as a refrigeration step to preserve the samples prior to extraction, minimizing enzymatic and oxidative changes during the processing interval.

2.3. Characterization of Tamarillo Puree Treated by FVE

2.3.1. Moisture

Moisture content was determined gravimetrically. A 1 g sample was placed in pre-weighed aluminum trays and dried in an oven at 105 °C for 24 h. The results were expressed as a percentage of moisture, in accordance with the AOAC [22].

2.3.2. Titratable Acidity and pH

Titrate acidity was determined as follows: approximately 5 g of puree were weighed, diluted in a known volume of distilled water, brought to 50 mL, and filtered. An aliquot of the filtrate was titrated with 0.01 N NaOH using phenolphthalein as an indicator until the appearance of a persistent pale pink color endpoint. Results were expressed as a percentage of citric acid equivalent. The pH was measured using a potentiometer.

2.3.3. Color Properties

Color properties were evaluated using a CR-400 colorimeter (Konica Minolta, Osaka, Japan). The results were interpreted according to the CIE Lab scale, used to calculate chroma (C^*), hue angle (h°), and total color difference (ΔE) using Equations (1), (2), and (3), respectively.

$$Chroma = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (1)$$

$$Hue\ angle = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (3)$$

where the subscript “0” indicates the value of the reference sample. L^* represents lightness, a^* the red-green coordinate, and b^* the blue-yellow coordinate.

2.3.4. Total Phenolic Content (TPC)

For the determination of total phenolic content (TPC), total anthocyanin content, and DPPH radical scavenging activity, extracts were obtained from the puree according to the following methodology: 20 g of puree were mixed with an 80% ethanolic solution in a 1:5 (m/v) ratio, the mixture was stirred for 1 h using a magnetic stirrer (hotplate stirrer) at controlled speed to ensure uniform dispersion. Then, the samples were subjected to ultrasonic treatment for 15 min, filtered under vacuum, and concentrated in a rotary evaporator (RV 10 Basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 35 °C under a vacuum pressure of 630 mmHg until a soluble solids content of $5 \pm 1\%$ was reached.

Total phenol content was determined using the Folin–Ciocalteu method described by Singleton et al. [23], with slight modifications: 0.5 mL aliquots of extract were mixed with 0.5 mL of concentrated Folin–Ciocalteu reagent and 7.5 mL of deionized water. The mixture was incubated at room temperature for 10 min, then combined with 1.5 mL of 20% (w/v) sodium carbonate solution. Finally, the sample was incubated for 1 h and measured at a wavelength of 760 nm using a UV-visible spectrophotometer (Evolution 260, Thermo Fisher Scientific, Waltham, MA, USA). Results were expressed as milligrams of gallic acid equivalents per gram of dry weight ($\text{mg GAE} \cdot \text{g}^{-1} \text{ D.W.}$).

2.3.5. Total Anthocyanin Content (TAC)

Total anthocyanin content was determined according to the methodology described by Giusti and Wrolstad [24]. 1 mL of concentrated extract was diluted in 4 mL of a pH 1.0 buffer solution, and the same procedure was repeated using a pH 4.5 buffer solution. After dilution, the samples were incubated in the dark for 20 min and subsequently measured at 520 and 700 nm using a UV-visible spectrophotometer (Evolution 260, Thermo Fisher Scientific, Waltham, MA, USA). Equation (4) was used to calculate the total anthocyanin content.

$$A = \frac{(A_{520} - A_{700})pH1 - (A_{520} - A_{700})pH4.5}{\epsilon \times 1} \quad (4)$$

where $A = (A_{520} - A_{700})_{\text{pH } 1} - (A_{520} - A_{700})_{\text{pH } 4.5}$, MW is the molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF is the dilution factor, and ϵ is the molar extension coefficient.

2.3.6. DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined following the method described by Brand-Williams et al. [25]. For the reaction, 3.9 mL of a 60 μM DPPH solution was mixed with 0.1 mL of extract. The mixture was vortexed and incubated at room temperature in the dark for 30 min. Absorbance was measured at 517 nm using a UV-visible spectrophotometer (Evolution 260, Thermo Fisher Scientific, Waltham, MA, USA). Results were expressed as μmol Trolox equivalents per gram of sample ($\mu\text{mol TE} \cdot \text{g}^{-1}$ D.W.), based on a Trolox calibration curve.

2.4. Preparation of Whey Protein-Based Hydrogels (WPH)

The WPH was prepared according to the methodology described by Bilek et al. [26], with slight modifications. A 20% (*w/v*) aqueous whey protein solution was prepared using deionized water. Subsequently, 33 mL of the protein solution was mixed with 5 g of extract ($^{\circ}\text{Brix } 5$). The solution was homogenized and adjusted to pH 1.5 using 3 M HCl, and then centrifuged at $5000 \times g$ for 5 min at 20 $^{\circ}\text{C}$ using a microcentrifuge (DLAB Scientific Co., Ltd., Beijing, China), after distributing the total volume into microcentrifuge tubes (Eppendorf-type) to separate insoluble fractions. The resulting supernatant was used to form the hydrogel via the heat-induced emulsification/gelation method. For this, it was mixed with soybean oil at 50 $^{\circ}\text{C}$ in a 1:5 (*v/v*) ratio (supernatant: soybean oil). The mixture was then heated from 50 to 80 $^{\circ}\text{C}$ over 6 min, then held at 80 $^{\circ}\text{C}$ for 10 min to allow gelation. Afterward, the suspension was cooled to 20 $^{\circ}\text{C}$ and centrifuged again under the same conditions. The oil phase was discarded, and the precipitated hydrogel was recovered and washed with 0.4% NaCl solution at pH 1.5, acidified with 1 M HCl. Finally, the samples were centrifuged and stored at 4 $^{\circ}\text{C}$ in amber glass bottles until analysis.

Generation of Whey Protein-Based Cryostructured Gel (WPCG)

Prior to freeze-drying, the WPH was immersed in liquid nitrogen (-196°C) for 60 min to ensure complete and uniform freezing of the hydrogel matrix. No observable structural damage was detected under these conditions. The samples were subsequently freeze-dried at -80°C under a vacuum pressure of 0.068 mbar for 48 h using a lyophilizer (FreeZone 4.5 Plus, Labconco Corp., Kansas City, MO, USA). After dehydration, the resulting whey protein-based cryostructured gel (WPCG) was stored under vacuum until analysis.

2.5. Characterization of Generated WPCG

2.5.1. Determination of Moisture Content and Water Activity (a_w)

The moisture content was determined according to the gravimetric method described by the AOAC [22]; a_w was measured at 25 $^{\circ}\text{C}$ using a water activity meter (Novasina, LabMaster, Lachen, Switzerland). Approximately 1 g of the sample was placed in the measurement chamber, and the instrument was calibrated prior to each determination using standard supersaturated salt solutions.

2.5.2. Bulk Density

The WPCG particles were carefully filled into a graded cylinder and weighed. The bulk density was then calculated from the particles' mass (m) and the sample bulk volume (v_b) (Equation (5)).

$$\rho_b = \frac{m}{v_b} \quad (5)$$

2.5.3. Porosity

The WPCG porosity (%) was estimated based on the following Equation (Equation (6)) [27].

$$\text{porosity (\%)} = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 \quad (6)$$

where ρ_b ($\text{g}\cdot\text{cm}^{-3}$) is the bulk density of the WPCG ρ_t ($\text{g}\cdot\text{cm}^{-3}$) is its true density (i.e., skeletal density). In particular, ρ_t was considered as WP true density ($\rho_t = 1.35 \text{ g}\cdot\text{cm}^{-3}$) [28].

2.5.4. Nitrogen Sorption Measurement

Measurements of the specific surface area (S_{BET}) and cumulative mesoporous surface area (S_{BJH}), were performed using an ASAP 2020 analyzer (Micromeritics Instrument Corp., Norcross, GA, USA). Prior to analysis, WPCG samples were degassed under vacuum and then analyzed at 77 K over a relative pressure (P/P°) range of 0.01–0.99, with approximately 32 data points recorded.

2.5.5. Scanning Electron Microscopy (SEM)

The microstructure of the cryostructured gels (WPCG) was examined by scanning electron microscopy (SEM). Samples in a dry state were gently mounted onto copper stubs using conductive adhesive. Prior to observation, the samples were sputter-coated with a gold–palladium layer (80:20) using a JFC-1100 ion sputter coater (JEOL, Tokyo, Japan) to ensure electrical conductivity.

The observations were carried out using a JSM-6390 scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 15 kV. Micrographs were digitally recorded for structural analysis.

2.5.6. Color Properties

Color properties were evaluated using CIE Lab parameters, hue angle, chroma, and total color difference (ΔE), as defined by Equations (1), (2), and (3), respectively.

2.5.7. TPC, TAMC, and DPPH Radical Scavenging Activity

WPCG generated was mixed with an acetone–water (70:30; 1% acetic acid) solution and vortexed for 2 min. The mixtures were placed in a shaker incubator operated at 50 °C and 200 rpm agitation for 60 min. Finally, the mixtures were centrifuged at $2500\times g$ for 10 min using a microcentrifuge (DLAB, Beijing, China). The supernatants were collected in tubes, and the extracts were used to determine TPC, TAMC, and DPPH radical scavenging activity, as described previously.

2.6. Statistical Analysis

All results were presented as mean values $\pm$ standard deviation. One-way analysis of variance (ANOVA) followed by the Tukey's test was performed to determine significant differences between treatments ($p < 0.05$). Statistical analysis was performed using Minitab software (version 17, Minitab, LLC, State College, PA, USA).

3. Results

3.1. Characterization of Tamarillo Purees Treated by Flash Vacuum Expansion

The characterization of FVE-treated tamarillo purees provides insight into how structural disruption influences the redistribution and retention of bioactive constituents within the fruit matrix. Moisture, acidity, color, and phenolic content are considered interconnected indicators to evaluate the effect of FVE on in-process resource retention.

3.1.1. Moisture, Titratable Acidity, and pH

FVE processing significantly affected moisture, titratable acidity (TA), and pH (Table 1). Moisture content decreased with increasing processing severity, from 90.83% in the control to 88.17%, 85.80%, and 85.06% for FVE_{5min}, FVE_{10min} and FVE_{15min}, respectively, with no significant differences between FVE_{10min} and FVE_{15min}. This reduction is attributed to partial water evaporation induced by rapid depressurization during the expansion stage, while the plateau observed at higher severity suggests rapid vacuum cooling and reduced flash-evaporation efficiency, as previously reported for expansion processes [12].

Table 1. Physicochemical and chemical parameters of tamarillo puree subjected to FVE process at different processing times.

Parameters	Control	FVE _{5min}	FVE _{10min}	FVE _{15min}
Moisture (%)	90.83 ± 0.86 ^a	88.17 ± 0.60 ^b	85.80 ± 0.29 ^c	85.06 ± 0.96 ^c
pH	2.12 ± 0.01 ^a	2.27 ± 0.01 ^b	2.32 ± 0.03 ^b	2.32 ± 0.01 ^b
Titratable acidity ¹	1.21 ± 0.01 ^a	1.49 ± 0.01 ^b	1.29 ± 0.009 ^b	0.71 ± 0.03 ^c
TAC ²	0.31 ± 0.009 ^a	0.75 ± 0.026 ^b	0.53 ± 0.057 ^c	0.24 ± 0.005 ^d
TPC ³	6.94 ± 0.28 ^a	11.68 ± 0.12 ^b	8.79 ± 0.21 ^c	5.52 ± 0.01 ^d
Radical Scavenging Activity ⁴	3.02 ± 0.08 ^a	4.35 ± 0.02 ^b	4.31 ± 0.04 ^b	4.35 ± 0.003 ^b
Color parameters				
L*	26.25 ± 0.42 ^a	28.71 ± 0.33 ^b	27.71 ± 0.33 ^c	25.03 ± 0.34 ^d
a*	18.09 ± 1.15 ^a	25.19 ± 0.85 ^b	23.95 ± 1.39 ^b	23.17 ± 1.71 ^b
Chroma	18.05 ± 1.15 ^a	25.14 ± 0.85 ^b	23.89 ± 1.41 ^b	23.10 ± 1.69 ^b
Hue angle	3.98 ± 0.33 ^a	3.67 ± 0.21 ^a	3.43 ± 0.55 ^a	4.27 ± 0.36 ^a
ΔE	-	7.53 ± 0.81	6.83 ± 0.09	5.24 ± 1.59

¹ Titratable acidity (g of citric acid·100 g⁻¹ FW); ² Total anthocyanin content (mg C3G·g⁻¹ DW); ³ Total phenol content (mg of GAE·g⁻¹ DW); ⁴ DPPH radical scavenging activity (μmol TEAC·g⁻¹ DW); FVE (Flash Vacuum Expansion). Different superscript letters (a–d) indicate significant differences between samples according to Tukey's multiple comparison test ($p < 0.05$). L* and a* correspond to the CIELAB color parameters (lightness, red–green coordinates, respectively).

Titratable acidity ranged from 0.71 to 1.49 g citric acid·100 g⁻¹ FW, values comparable to those reported for red and purple tamarillo varieties (1.38–1.46 g citric acid·100 g⁻¹ FW) [29]. FVE_{5min} and FVE_{10min} treatments significantly increased TA relative to the control, whereas FVE_{15min} resulted in a marked decrease. The initial increase is consistent with enhanced release of organic acids due to tissue disintegration and the migration of compounds from the peel, as reported for FVE-treated mangoes and red wine matrices [30,31]. This behavior is associated with the disruption of vacuoles—major storage sites of organic acids—which promotes their release into the matrix [32]. In contrast, the decrease at higher severity suggests partial degradation or transformation of organic acids under prolonged thermal exposure. A similar trend was observed between TA and total phenolic content (TPC), as discussed later, supporting previously reported associations between acidity, phenolics, and antioxidant activity [33].

FVE induced a significant increase in pH of approximately 7–9% across treatments, from 2.12 in the control to approximately 2.32 in treated samples. Comparable increases have been reported in other fruit systems processed by flash expansion, including passion fruit (up to 18%) [34] and *Physalis peruviana* purees (7–9%) [35]. These changes have been attributed to tissue disruption and redistribution of organic acids within the matrix, leading to compositional re-equilibration, which is consistent with the structural and compositional modifications observed in the present study.

3.1.2. Color Properties of Tamarillo Purees

Color is one of the most important quality attributes to be evaluated during fruit processing. It represents the consumer's first visual impression of the product and can therefore influence its acceptance or rejection, particularly when color changes are associated with quality deterioration or spoilage.

The effect of FVE processing on color properties has been widely reported for fruits such as passion fruit, grapes, blackberries, mangoes, avocados, and papayas, where FVE-treated products show significant enhancements in color attributes compared with conventional technologies, such as maceration or enzymatic treatments. These improvements contribute to the production of products with superior visual quality [12,34–37].

The visual appearance of FVE-processed tamarillo purees is presented in Table 2, while Table 1 summarizes the color parameters obtained from instrumental color analysis.

Table 2. Visual appearance of tamarillo purees before and after FVE treatment.

Treatment	Visual Appearance
Control	
FVE _{5min} = 65 °C	
FVE _{10min} = 77 °C	
FVE _{15min} = 91 °C	

In the present study, the a^* parameter (red–green axis) ranged from 18.09 to 25.19. These values are fully consistent with those reported for red tamarillo varieties (18.0–25.7), confirming that FVE treatment preserved the characteristic chromatic profile of the fruit within its natural variability. FVE_{5min} and FVE_{10min} significantly increased a^* (39% and 32%, respectively), indicating a shift toward redder tones. This behavior is consistent with the higher TAC concentrations (discussed later) observed under these conditions and agrees with reports describing a positive relationship between anthocyanin content and a^* values for Grenache, Carignan, Mourvèdre, and Pinot Noir grapes treated by FVE [13,31].

Under the most severe condition (FVE_{15min}), a^* remained higher than the control despite a reduction in TAC. This suggests that structural modifications also contributed to color changes. It is well established that food structure and pigmentation interact, and structural changes may alter internal light scattering and surface reflectance, leading to color variations that are not necessarily associated with pigment concentration [38,39]. Textural changes, such as variations in the viscosity of passion fruit purees processed by FVE at 95 °C under similar conditions to those of the FVE_{15min} treatment, were previously reported by Brat et al. [34].

Chroma increased markedly at FVE_{5min}, indicating enhanced color saturation, and remained significantly higher than the control at FVE_{10min} and FVE_{15min} treatments. In contrast, hue angle did not show statistically significant differences among treatments ($p > 0.05$), with mean values ranging from 3.43° to 4.27°. Although minor numerical variations were observed, these differences were small and remained within a narrow angular interval characteristic of red-dominated chromatic coordinates. The consistently low hue values confirm that the color of tamarillo purees remained strongly oriented toward the red region of the CIELAB space, indicating that FVE processing did not alter the dominant chromatic tone. Therefore, chroma exhibited greater sensitivity to FVE severity than hue angle under the evaluated conditions.

Total color difference (ΔE) was significantly affected by processing conditions, with the largest variations observed at FVE_{5min} and FVE_{10min}. Although ΔE decreased at FVE_{15min}, all treated samples exhibited values above 5, indicating visually perceptible color differences [40].

Overall, mild to moderate FVE conditions enhanced redness and chromatic intensity, whereas higher severity limited further improvement, likely due to structural effects rather than additional pigment release. From a resource valorization perspective, the preservation and enhancement of chromatic attributes under moderate FVE conditions is relevant, as visual quality directly influences product acceptability and marketability. Maintaining color intensity without extensive thermal degradation may help reduce quality-related losses and improve the effective utilization of tamarillo biomass in value-added formulations.

3.1.3. Total Phenolic Content, Total Anthocyanin Content, and Radical Scavenging Activity by DPPH

The FVE process modulated total phenolic content (TPC), total monomeric anthocyanins (TAC), and radical scavenging activity (RSA) in a coordinated manner, evidencing a temperature–time dependent response that simultaneously involved compound release and partial degradation (Table 1).

TPC values ranged from 6.94 to 11.68 mg GAE·g^{−1} DW, which falls within the previously reported interval for red tamarillo fruits grown in Malaysia and India (2.53–15.23 mg GAE·g^{−1} DW) [11,41]. Similarly, TAC values (0.24–0.75 mg cyanidin-3-glucoside·g^{−1} DW) exceeded those reported for red and red-purple tamarillo cultivated in Inter-Andean Valley of Ecuador and Northwest Argentina (0.01–0.62 mg cyanidin-3-glucoside·g^{−1} DW) [4,42,43]. Such variability may be attributed to differences in maturity stage, genotype, or environmental conditions [1], but also reflects the impact of FVE processing.

Under moderate processing conditions (FVE_{5min} and FVE_{10min}; 65–77 °C), TPC increased by 68.29% and 26.65%, respectively, while TAC rose by 141% and 70%, compared to the control. This parallel enhancement was accompanied by an $\approx 49\%$ increase in RSA (from 3.02 to ≈ 4.3 $\mu\text{mol TEAC}\cdot\text{g}^{-1}$ DW), suggesting that phenolic enrichment contributed directly to the antioxidant response. A similar enrichment of anthocyanins and phenolics following FVE has been reported in grape juices and passion fruit beverages [12,35], as well as in mango purees [30,37].

Mechanistically, this increase can be attributed to two complementary phenomena. First, FVE promotes microstructural and cellular disintegration through microchannel formation [34], facilitating the migration of phenolic compounds from the peel into the puree. This is relevant considering that red tamarillo peel contains substantially higher phenolic and anthocyanin concentrations (4.08–16.73 mg GAE·g⁻¹ for phenolics; 1.36–1.55 mg·g⁻¹ for cyanidin-3-glucoside) [1,5,43]. Second, instantaneous water evaporation during de-pressurization induces vacuolar rupture [44], enhancing the release of compartmentalized phenolics. Comparable compound enrichment due to expansion-driven cellular disruption has been described in mangoes and grape matrices processed by FVE [12,30,37].

Importantly, although peel and seed residues were mechanically separated after puree recovery (as described in the methodology), the fruit was processed intact during FVE treatment. This sequence allows structural disruption and internal redistribution of phenolic-rich peel constituents prior to mechanical separation. Therefore, the observed increases in TPC and TAC in the recovered puree cannot be attributed solely to the compositional inclusion of peel tissue, but rather to FVE-induced microstructural modifications that promote the migration of bioactives before the fraction is discarded.

When contextualized against conventional thermal treatments applied to tamarillo matrices, the magnitude and statistical significance of the FVE-induced increases become particularly relevant. Steam cooking at 100 °C for 5 min has been reported to increase TPC by approximately 56%; however, this change was not statistically significant ($p > 0.05$) and was evaluated against a freeze-dried reference matrix rather than a fresh control [45]. Conventional water blanching at 85 °C for 3 min resulted in a slight reduction (~5%) in TPC relative to fresh fruit, without significant differences [46]. These findings suggest that thermal softening alone does not consistently translate into statistically validated enhancement of phenolic recovery in tamarillo systems.

In contrast, the increases observed under FVE_{5min} (68.29% for TPC and 141% for TAC) and FVE_{10min} (26.65% for TPC and 70% for TAC) were statistically significant relative to the control, even after post-process separation of peel residues. Although the magnitude of enhancement was lower at FVE_{10min} compared to FVE_{5min}, statistical significance confirms that redistribution and recovery mechanisms remain operative under moderate thermal severity. From a resource valorization perspective, this indicates that FVE enhances in-process recovery of phenolic compounds before unavoidable mechanical fractionation, thereby reducing early-stage losses typically associated with conventional peel-removal workflows.

When processing severity increased (FVE_{15min}; ≈91 °C), a marked reduction in phenolic-related parameters was observed. TPC decreased by 20.46% relative to the control, and TAC exhibited a 22% reduction. These losses are consistent with reports describing phenolic degradation (19–32%) in heat-treated apple and jabuticaba [47,48], as well as the thermolabile nature of anthocyanins, which may experience 28–80% losses at ≈90 °C [49,50]. In agreement with Morel-Salmi et al. [13], increased temperature during FVE may initially favor extraction but can subsequently promote pigment conversion or oxidative reactions.

Interestingly, despite the reduction in TPC and TAC at FVE_{15min}, RSA remained significantly higher than the control (4.35 μmol TEAC·g⁻¹ DW). No statistically significant differences were observed among FVE_{5min}, FVE_{10min}, and FVE_{15min} treatments ($p > 0.05$), indicating that antioxidant activity remained comparably elevated across all FVE conditions. This behavior suggests that antioxidant activity is not exclusively governed by total phenolic concentration. Tamarillo also contains other antioxidant constituents, such as ascorbic acid and carotenoids, which may sustain redox activity even when phenolic content decreases. Indeed, Espin et al. [51] reported higher antioxidant activity in tamarillo

than in kiwi and grape, despite similar or lower phenolic concentrations, reinforcing the concept of a multifactorial antioxidant system.

Overall, these results indicate that moderate FVE conditions enhance bioactive extractability beyond levels reported for conventional thermal softening alone, while minimizing fractionation-related exclusion of phenolic-rich tissues. Rather than relying on complete tissue retention, FVE improves redistribution efficiency within the intact fruit prior to separation, strengthening the argument for in-process resource retention within tamarillo value chains.

Accordingly, FVE_{5min} was selected as the optimal condition, as it required the shortest processing time while providing the highest concentrations of functional compounds and the most favorable color attributes. FVE_{5min} was used for the encapsulation in WPH.

Collectively, these findings indicate that FVE influences multiple quality attributes in a coordinated manner while enhancing statistically validated recovery of phenolic-rich fractions from the fruit matrix prior to mechanical fraction discard. This integrated response supports the interpretation of FVE as a structural processing strategy that strengthens in-process resource retention within tamarillo-based value chains.

3.2. Characterization of the WPH and WPCG

3.2.1. Moisture Content and Water Activity (a_w)

The results for moisture and water activity (a_w) reflect the physical and thermodynamic transitions occurring during the cryostructuring process, in which solvent removal by sublimation transforms a highly hydrated polymer network into a dehydrated solid matrix. This transition is particularly relevant because residual moisture and water activity directly influence the physicochemical stability of the encapsulated tamarillo bioactive compounds.

The WPH moisture content, equivalent to $86 \pm 0.43\%$, is consistent with whey protein gels, in which a three-dimensional hydrophilic network retains large amounts of water [52]. In contrast, the moisture content of the cryostructured gel, $5.21 \pm 0.94\%$, falls at the lower end of the ranges reported for similar WPI-based systems (5–20%) [52], indicating an efficient freeze-drying process that promoted effective water removal from the matrix.

Such a marked reduction in residual moisture is technologically significant because lower water availability restricts molecular mobility and reduces the probability of hydrolytic and oxidative reactions that could compromise the stability of phenolic compounds recovered from tamarillo. Therefore, the dehydration achieved through cryostructuring directly stabilizes the recovered bioactive fraction within the protein-based matrix.

In terms of a_w , the reduction from 0.81 ± 0.01 in the WPH to 0.17 ± 0.01 in the cryogel is critical for system stability. A water activity of 0.81 implies high molecular mobility and availability of free water, conditions associated with increased rates of hydrolysis and degradation of bioactive compounds [53].

In contrast, the value of 0.17 in the cryostructured gel (WPCG) is notably low and is lower than those reported for other lyophilized encapsulation systems [54]. At such low a_w levels, the availability of free water is extremely limited, as water is predominantly associated with the polymer matrix rather than acting [55]. This condition is generally unfavorable for the occurrence of many chemical reactions and for microbial growth [56]. Under these conditions, the system can be interpreted as approaching a glassy-like state, where the reduced segmental mobility of the protein network limits the probability of reactive molecular interactions [55,57].

Additionally, the dense polymer walls formed during cryostructuring act as physical barriers, imposing diffusion limitations on both oxygen and residual water, further reducing the rates of oxidative and hydrolytic reactions [55,58].

Such low a_w values are therefore technically desirable, as they not only limit molecular mobility [53], but also contribute to restricting reaction kinetics and enhancing the physico-chemical stability of the encapsulated fraction. From a resource stabilization perspective, achieving an a_w of 0.17 minimizes secondary quality losses during storage and supports more efficient utilization of the phenolic-rich tamarillo fraction.

3.2.2. Structural Properties of WPCG (Bulk Density, Porosity, and Surface Area)

Bulk density, porosity, and specific surface area are critical structural parameters in the characterization of cryogels, as they influence mass transport, liquid absorption and retention, and mechanical stability [59]. In cryostructured materials, these properties are primarily governed by the porous architecture formed during freezing, in which ice crystal formation serves as a templating mechanism prior to lyophilization.

The low bulk density values obtained for WPCG ($0.109 \pm 0.02 \text{ g}\cdot\text{cm}^{-3}$), together with its high porosity ($91.90 \pm 0.03\%$), classify this matrix as an ultra-low-density and highly porous material within the range reported for supermacroporous cryogels [18,60]. Such values indicate a high void fraction capable of accommodating dispersed phases within an interconnected network. In comparison, Ciuffarin et al. [17] reported an approximate density of $0.19 \text{ g}\cdot\text{cm}^{-3}$ and a porosity close to 85% for protein concentrate cryogel particles frozen at -40°C prior to lyophilization, suggesting a lower void fraction. Similarly, investigations on protein and polysaccharide cryogels prepared under conventional freezing (i.e., -40 to -20°C) or immersion in liquid nitrogen show densities typically between 0.06 and $0.29 \text{ g}\cdot\text{cm}^{-3}$, depending on the freezing conditions [17,59], highlighting the sensitivity of bulk structural properties to thermal history.

The observed structural features in WPCG are consistent with the use of ultra-rapid freezing in liquid nitrogen (-196°C), which is generally expected to promote high nucleation rates and limit extensive crystal growth [18]. According to Meseldzija et al. [59], rapid cooling reduces the time available for coalescence and crystal growth, often resulting in a more homogeneous pore network after sublimation. In contrast, slow freezing promotes directional crystal growth and the formation of larger ice crystals, producing coarser and more heterogeneous pores and, in some cases, structures with higher bulk density due to partial reorganization or local collapse of the network [59,60]. While the present study did not directly measure ice crystal size or freezing rate, the resulting pore morphology observed by SEM (Figure 1A) is consistent with previously reported cryostructured architectures produced under rapid freezing conditions.

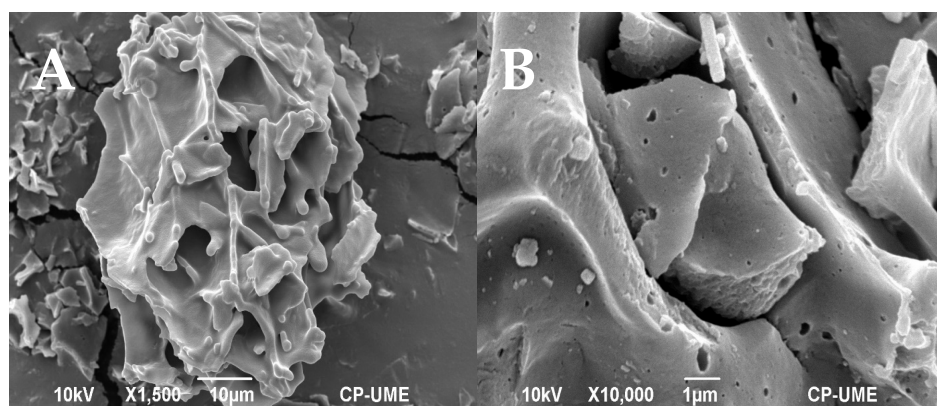


Figure 1. Scanning electron micrographs of the whey protein-based cryostructured gel: (A) the general interconnected macroporous structure with large open pores ($\times 1500$); (B) surface texture of pore walls ($\times 10,000$).

In cryostructured systems, ice crystals act as structural templates (porogens), whose removal by sublimation preserves the spatial organization of the previously consolidated polymer network, a phenomenon described by Lozinsky [14] as cryostructuring. Thus, although rapid freezing can generate smaller individual pores than slow freezing, the high initial ice volume fraction and the preservation of the interconnected architecture contribute to a high total void volume, which explains the low density observed in WPCG. The SEM micrographs (Figure 1A) reveal open cavities at the micrometer scale and a continuous three-dimensional network, features that have also been documented in starch- and alginate-based cryogels prepared under rapid freezing conditions [18].

Despite the high overall porosity ($91.90 \pm 0.03\%$), the specific surface area (SBET) was $0.32 \pm 0.01 \text{ m}^2 \cdot \text{g}^{-1}$. The similarity to BJH values ($0.24 \pm 0.01 \text{ m}^2 \cdot \text{g}^{-1}$ calculated from the adsorption isotherm of N_2) suggests that the accessible mesoporous fraction is minimal and that the structure is dominated by interconnected macropores. This apparent discrepancy is expected in cryostructured systems, where the BET method primarily quantifies micro- and mesoporosity, while the macropores generated as ice replicas contribute little to surface area measured by gas adsorption [19]. SEM analysis (Figure 1B) further supports this interpretation, showing compact pore walls without evidence of a developed mesoporous network.

Importantly, although high macroporosity implies a large open void fraction, the low water activity previously reported for WPCG ($a_w = 0.17 \pm 0.01$; Section 3.2.1) significantly limits molecular mobility and reduces the likelihood of hydrolytic or microbial deterioration within the matrix.

Therefore, structural openness does not necessarily translate into increased chemical instability under low-moisture conditions.

In this context, the combination of ultra-low density, high interconnected porosity, and reduced a_w defines a dehydrated architectural scaffold capable of immobilizing and stabilizing incorporated bioactives within a solid matrix.

From a resource valorization perspective, this architectural configuration enables the incorporation of tamarillo-derived bioactives into a lightweight, solid system without requiring prior solvent-based fractionation. By operating as a structural stabilization platform rather than a dense encapsulating shell, the cryostructured matrix supports in-process retention of redistributed bioactive compounds while maintaining physicochemical stability.

3.2.3. Color Properties of WPH and WPCG

The evolution of the color parameters (L^* , a^* , Chroma, and Hue angle) during the three processing steps reflects both pigment modifications during gelation and optical scattering effects associated with the porous structure formed during cryostructuring.

During gelation, L^* , a^* , and Chroma did not show statistically significant differences ($p > 0.05$), although a moderate overall color difference was observed ($\Delta E = 3.38 \pm 0.20$). The puree exhibited low lightness and a pronounced red component, consistent with its anthocyanin content. The partial reduction in anthocyanins (~48%) indicates that compositional modifications occurred during gelation and may contribute to the observed chromatic modulation.

The most pronounced change occurred during cryostructuring (from WPH to WPCG). Lightness increased significantly ($L^* = 52.48 \pm 1.66$), while a^* and Chroma decreased to 18.06 ± 0.53 and 17.43 ± 0.51 , respectively, resulting in a large total color difference relative to the puree ($\Delta E = 25.02 \pm 1.45$). This behavior is not consistent with substantial additional compositional losses during cryostructuring. Instead, the dominant contribution arises from water removal and the formation of an air-filled porous architecture, where sublimation of ice crystals produces interconnected supermacropores [16].

The increase in air–solid interfaces enhances multiple light scattering events, thereby increasing L^* . At the same time, this produces an apparent chromatic dilution effect that reduces saturation. Such behavior is consistent with optical responses described for dehydrated porous solids, where replacement of a continuous aqueous phase ($RI \approx 1.33$) by air-filled voids ($RI \approx 1.0$) increases refractive index contrast and promotes diffuse reflectance [61]. This optical response is associated with the increased refractive index contrast between the protein matrix and internal air voids generated during freeze-drying, rather than drastic compositional breakdown.

Hue angle increased progressively and significantly (from 3.66 ± 0.21 to 9.31 ± 0.11 and 14.50 ± 0.26), indicating a measurable angular displacement while remaining within the same red chromatic domain. Although statistically significant, this variation represents tonal modulation rather than a shift toward a different color family, suggesting that structural reorganization and partial pigment redistribution outweigh severe chromophore degradation.

Overall, the supermacroporous architecture generated by cryostructuring contributes to the optical response of the system, but its effect is coupled with prior pigment modification occurring during gelation, resulting in a lighter and less saturated matrix while preserving a high proportion of phenolic compounds.

3.2.4. Total Phenolic Content, Total Anthocyanin Content and DPPH Radical Scavenging Activity

In accordance with the supermacroporous architecture previously described for WPCG, the reduction in bioactive compounds occurred predominantly during the WPH formation stage rather than during cryostructuring. Total phenol content showed a retention of 84.3% after gelation (9.85 ± 0.24 vs. 11.68 ± 0.12 mg of GAE·g^{−1} DW) and 83.1% in the cryostructured gel, confirming that the transition from hydrogel to cryostructured gel did not result in statistically significant ($p > 0.05$) additional losses. Similarly, DPPH radical scavenging activity showed a retention of 86.0% in the WPH (3.74 ± 0.16 vs. 4.35 ± 0.01 μmol TEAC·g^{−1} DW) and 84.1% in the WPCG (3.65 ± 0.31 vs. 4.35 ± 0.01 μmol TEAC·g^{−1} DW), demonstrating a non-significant ($p > 0.05$) additional decrease associated with the freezing and lyophilization steps.

In contrast, anthocyanins exhibited greater susceptibility, with retentions of 48.29% after gelation (0.36 ± 0.01 vs. 0.75 ± 0.02 mg cyanidin-3-glucoside·g^{−1} DW) and 46.21% in the cryostructured gel (0.34 ± 0.01 vs. 0.75 ± 0.02 mg cyanidin-3-glucoside·g^{−1} DW), reflecting their well-known sensitivity to processing conditions, including temperature variations and alterations in the protein microenvironment. The degradation of anthocyanins during the production of whey protein-based hydrogels is consistent with what has been reported in the literature [26].

When contextualized against conventional dehydration processes applied to tamarillo, similar sensitivity patterns have been reported. Spray-dried tamarillo powders processed at inlet temperatures around 130 °C have shown anthocyanin retentions of approximately 58% [62], indicating that partial degradation during stabilization is not exclusive to protein-based systems but characteristic of thermally assisted preservation routes. Importantly, in spray drying, apparent increases in phenolic concentration per dry weight basis often result from water removal rather than absolute retention of the original phytochemical stock [6].

These total phenol retention percentages (≈ 83 – 84% for WPH and WPCG, respectively) fall within the range reported for whey protein hydrogel encapsulation systems. Bilek et al. [26] reported phenolic compound retentions between 70 and 85% after incorporating anthocyanin-rich extracts into WPI hydrogels, attributing the initial decrease to protein–polyphenol interactions and structural rearrangements associated with thermal denaturation, rather than to extensive oxidative degradation. A strong correlation between

total phenolic content and antioxidant activity has consistently been reported in tamarillo fruit [29], supporting the conclusion that the observed decrease in DPPH in the present study is primarily due to a reduction in the extractable or chemically accessible phenolic fraction, rather than to complete degradation.

From a mechanistic perspective, during thermal gelation, a three-dimensional network of partially denatured proteins forms, in which phenolic hydroxyl groups can establish non-covalent interactions (hydrogen bonds, hydrophobic interactions, and electrostatic interactions) with protein chains [63]. This phenomenon may reduce extractability and chemical accessibility toward the DPPH radical without necessarily implying complete degradation of the phenolic compounds. Subsequently, the cryostructuring step—governed by the dynamics of ice crystallization and sublimation [16]—reorganizes the already consolidated network into a supermacroporous architecture, without introducing significant additional thermal stress. The low specific surface area (SBET) and the limited micro/mesoporous fraction [19] support the interpretation that rapid freezing acts predominantly as a physical templating mechanism for pore generation, which explains the relative stability of phenols and antioxidant activity during this stage [53].

Unlike spray-dried powders, which are formed as discrete glassy microparticles, typically dependent on carrier solids to ensure structural stability [54], the cryostructured system yields a continuous three-dimensional scaffold in which redistributed solutes are physically immobilized within interconnected supermacropores. This architectural distinction does not eliminate losses during gelation; however, it defines a mechanistically distinct stabilization pathway based on structural immobilization rather than a rapid glass transition.

Overall, the WPCG showed total phenol retention greater than 83% and an antioxidant activity retention close to 84%, values comparable to those reported in the literature for protein hydrogels, where retention percentages of approximately 75% have been described during the entrapment of black carrot anthocyanins in whey protein hydrolysate-based hydrogels [26]. The greater reduction observed for anthocyanins confirms their higher structural sensitivity.

At the process level, the significance of this system lies not in preventing degradation—since partial losses are observed across most dehydration and encapsulation strategies—but in integrating biomass redistribution and structural immobilization within a single processing sequence. Following intracellular release, phenolic compounds are confined within a continuous supermacroporous scaffold, preserving more than 80% of the total phenolic fraction inside the primary matrix.

This stabilization route differs from carrier-assisted spray drying, where rapid water removal and structural stabilization in spray drying are strongly dependent on the formation of a glassy matrix, typically achieved through the addition of high-glass transition temperature carrier solids such as maltodextrin or Gum Arabic [54]. In the present system, immobilization is governed by architectural confinement within a protein-based network, resulting in a continuous porous matrix rather than discrete particles. Such differentiation does not imply superior retention; however, it defines an alternative structural format that may enable distinct incorporation modes in semi-solid or solid food systems where spatial distribution and volumetric integrity are relevant.

4. Conclusions

The combined disruption–stabilization sequence demonstrated that intracellular redistribution of tamarillo phytochemicals can be effectively coupled with architectural immobilization within a protein-based scaffold, resulting in retention of more than 80% of

total phenolics and antioxidant activity, with no statistically significant additional losses during cryostructuring.

Although partial anthocyanin reduction occurred during gelation, the subsequent stabilization stage did not intensify compositional degradation. The marked decrease in water activity ($a_w \approx 0.17$) and the formation of a porous scaffold limited molecular mobility, supporting the physicochemical stability of the redistributed fraction. Optical modifications observed after freeze-drying were primarily associated with water removal and structural light scattering rather than drastic pigment breakdown.

From a process-level perspective, integrating controlled biomass disruption with structural immobilization reduced early-stage exclusion and confined redistributed phytochemicals within the primary product matrix.

This approach emphasizes retention of initial phytochemical stocks within the processing stream, offering a structurally differentiated stabilization pathway relative to conventional atomization-based routes.

Among the evaluated conditions, FVE_{5min} was identified as the most suitable pre-treatment, offering a favorable balance between structural modification and retention of bioactive compounds, supporting its use for the preparation of WPH and WPCG.

Overall, the findings indicate that coupling redistribution and architectural confinement within a unified sequence can improve the utilization of tamarillo biomass fractions in agri-food systems.

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