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Blackberry Juice Concentrated by Nanofiltration: Characterization, Stability and Application in a Fruit Juice

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Abstract

Blackberry (*Rubus* spp.) is a highly perishable fruit rich in bioactive compounds, particularly anthocyanins, which are associated with significant health benefits. This study investigated the application of nanofiltration using a pilot-scale spiral-wound module (DOW® NF90-2540) as a mild technology to concentrate phenolic compounds, especially anthocyanins, in blackberry juice. The process achieved concentration factors (CF) of 2.2 for monomeric anthocyanins and 1.9 for total phenolic content (TPC), reaching values of 54.3 mg C3G·100 mL^{-1} and 326.85 mg GAE·100 mL^{-1}, respectively. The antioxidant capacity ($ABTS^{+}$ and DPPH methods) also increased significantly in the concentrated fraction (CF 1.9 and 1.7, respectively). Stability of the concentrated juice was evaluated during 90 days of frozen storage, showing that low temperatures effectively preserved anthocyanin levels and visual quality, with only minor variations in color parameters (L*, a*, b*). Furthermore, the concentrated blackberry juice was successfully incorporated into apple–orange juice blends, generating formulations with progressively increased phenolic content, antioxidant activity, and red color intensity as the proportion of blackberry concentrate increased. Anthocyanin bioaccessibility in these juice blends was also evaluated and was not proportional to the increase in anthocyanin content. Strong correlations between anthocyanin concentration, antioxidant capacity, and CIELAB color parameters highlight the dual functional and technological role of blackberry compounds. In conclusion, this study demonstrates the feasibility of nanofiltration as a mild and efficient strategy for concentrating anthocyanins and phenolic compounds from blackberry juice while preserving physicochemical quality and color attributes.

Keywords: *Rubus* spp.; membrane separation; phenolic compounds; non-thermal processing; bioaccessibility

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1. Introduction

Red berries are widely consumed worldwide due to their attractive color, characteristic flavor, and high content of bioactive compounds, which contribute to both sensory quality and nutritional value [1]. Among these fruits, blackberry (*Rubus* spp.) has attracted increasing scientific and industrial interest owing to its high nutritional density, low caloric value, and richness in polyphenolic compounds, which are strongly associated with health-promoting effects [2].

From a phytochemical perspective, blackberry fruits present a complex composition, including phenolic acids, flavonoids, tocopherols, and carotenoids [3]. Anthocyanins are the predominant flavonoids and the main pigments responsible for the characteristic dark coloration of the fruit. These compounds are widely recognized for their antioxidant capacity and potential functional properties, contributing to the prevention of oxidative stress-related disorders [4]. During fruit ripening, anthocyanin accumulation increases markedly, with cyanidin-3-O-glucoside identified as the major anthocyanin in different cultivars, which directly correlates with enhanced antioxidant activity [5].

Recent studies have demonstrated that the antioxidant potential of blackberry is not solely attributed to anthocyanins but rather to a synergistic interaction among different phenolic classes. Ellagitannins, such as lambertianin C and sanguiin H-6, as well as flavan-3-ols, including epicatechin and procyanidin B2, significantly contribute to the overall antioxidant capacity of the fruit matrix, even after processing [6]. However, industrial processing steps may alter the phenolic profile, affecting color stability, antioxidant capacity, and sensory quality, highlighting the need for mild and selective processing technologies.

Despite its high functional value, blackberry is a highly perishable fruit, characterized by fragile texture, rapid physiological deterioration, and high susceptibility to microbial spoilage, which limits its shelf life and commercialization for fresh consumption [7]. Consequently, a substantial portion of blackberry production is directed toward industrial processing into juices, concentrates, purees, and functional ingredients, where the preservation of anthocyanins is crucial for product quality and consumer acceptance [8].

In this context, membrane separation technologies have emerged as effective alternatives to conventional thermal concentration processes in the food industry. These technologies operate under mild temperature conditions, allowing selective separation based on molecular size and physicochemical interactions, while minimizing thermal degradation of bioactive compounds and reducing energy consumption [9,10]. Among these processes, nanofiltration stands out for its ability to selectively retain compounds of intermediate molecular weight, such as polyphenols and anthocyanins, while allowing the passage of water and low-molecular-weight solutes [11]. However, the application of nanofiltration in acidic fruit matrices presents operational challenges, particularly regarding membrane stability and long-term durability under low-pH conditions, which may ultimately affect process performance and membrane lifespan [12].

Several studies have reported the successful application of nanofiltration in the concentration of wines, fruit juices, and plant extracts, demonstrating high retention of phenolic compounds and pigments with limited impact on product quality [9,13]. To the best of our knowledge, no previous study has evaluated nanofiltration of blackberry juice at pilot scale while simultaneously integrating membrane process performance with product stability, technological applicability in blended juices, and anthocyanin bioaccessibility.

Therefore, the aim of this study was to investigate the application of nanofiltration as a strategy to concentrate anthocyanins in blackberry juice, evaluating its efficiency and effects on physicochemical characteristics, as well as the potential use of the obtained concentrate in the formulation of apple and orange juice blends, aiming to increase their antioxidant compound content.

2. Materials and Methods

Figure 1 summarizes the experimental design adopted in this study.

Blackberry pulp was centrifuged and concentrated by nanofiltration, generating a retentate juice enriched in phenolic compounds and anthocyanins. This concentrate was incorporated into fruit juice blends containing apple and orange juices in different proportions. Following pasteurization, the samples were analyzed in triplicate for physicochemical

parameters, antioxidant activity, and color attributes, and then subjected to simulated gastrointestinal digestion according to the INFOGEST protocol. The bioaccessible fraction was collected for further analysis of bioactive compounds. Detailed information on each step of the study is provided in the following sections.

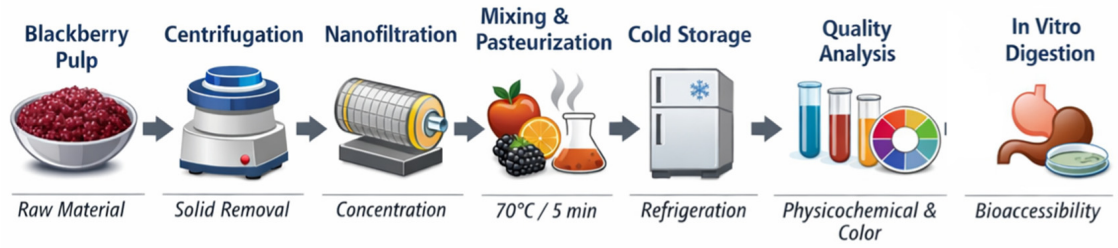

Figure 1. The experimental design adopted in this study.

2.1. Blackberry Juice Preparation

Frozen whole blackberry pulp (De Marchi®, São Paulo, Brazil) was purchased from a local distributor in Rio de Janeiro, Brazil. The pulp was thawed at refrigeration temperature, homogenized, and centrifuged using a basket centrifuge (IEC Centrifuge, Model K 7165, International Equipment Company, Needham, MA, USA) to reduce suspended solids and facilitate the subsequent nanofiltration process. The clarified juice was transferred to plastic containers and stored at −18 °C until further use.

2.2. Nanofiltration Process

Nanofiltration was carried out using a pilot-scale membrane system equipped with a spiral-wound module (effective membrane area of 2.0 m^2) fitted with a polyamide membrane (DOW® NF90-2540, DOW FILMTEC™, Twin Lakes, WI, USA), characterized by NaCl rejection higher than 97%. The system consisted of a feed tank containing the centrifuged blackberry juice, a recirculation pump, and pressure control devices, including valves and manometers (Figure 2).

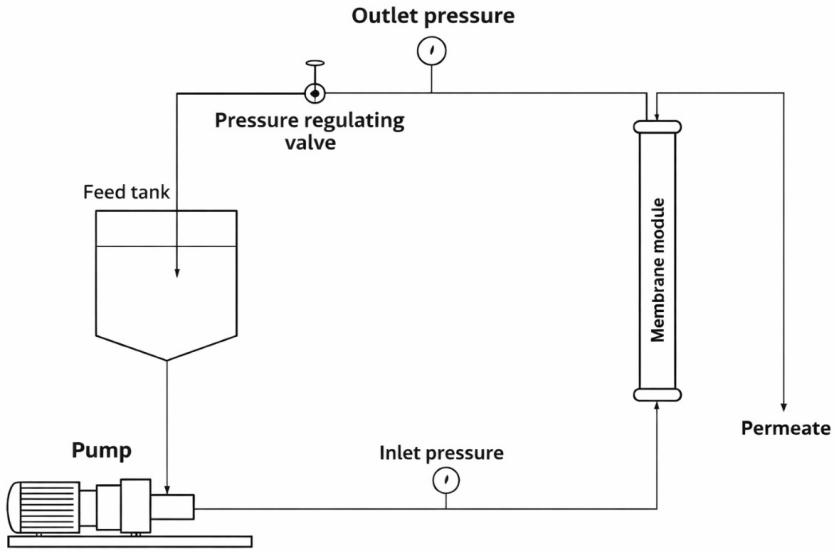

Figure 2. Nanofiltration process system for concentrating blackberry juice.

The process was conducted in batch mode with total recirculation of the retentate, while permeate was continuously collected. Operating conditions were maintained at a transmembrane pressure of 10 bar, equilibrium temperature of approximately 40 °C, and circulation flow rate of 817.2 L h^{-1}. This temperature resulted from pump operation and fluid recirculation, with no external heating applied.

2.3. Juice Characterization

2.3.1. Physicochemical Analyses

The pH was measured at 25 °C using a calibrated digital pH meter (MetrohmAG, Canton of Appenzell Ausserrhoden, Switzerland), with standard buffer solutions at pH 4.0 and 7.0. Titratable acidity was determined by potentiometric titration with standardized NaOH solution using an automatic titrator (Metrohm, Herisau, Switzerland) and expressed as g of citric acid per 100 g of sample. Soluble solids content (°Brix) was measured using an Abbe refractometer (Bellingham + Stanley). Total solids were determined gravimetrically after drying the samples in a vacuum oven at 80 °C until constant weight, according to AOAC (2016) [14].

Color parameters (L*, a*, b*) were determined by reflectance using a colorimeter operating in the CIELAB system ColorQuest XE(Hunter Associates Laboratory, Inc, Fairfax, VA, USA). The instrument was calibrated with a white standard supplied by the manufacturer prior to measurements. Analyses were performed in triplicate.

2.3.2. Total Phenolic Compounds

Total phenolic content was determined using the Folin–Ciocalteu method, as described by Singleton and Rossi (1965) [15] and modified by Georgé et al. (2005) [16]. After 2 min of reaction with the Folin reagent, sodium carbonate solution (7.5%, w/v) was added, and the mixture was incubated in a water bath at 50 °C for 15 min. Absorbance was measured at 760 nm using a UV–Vis spectrophotometer, with 95% ethanol as the blank. Quantification was based on a gallic acid calibration curve (0–100 mg·L^{-1}), and results were expressed as mg gallic acid equivalents per 100 mL of sample (mg GAE·100 mL^{-1}).

2.3.3. Total Monomeric Anthocyanins

Total monomeric anthocyanin content was determined by the pH differential method, according to Fuleki and Francis (1968) [17], as modified by Giusti and Wrolstad (2001) [18]. Samples were diluted in potassium chloride buffer (0.025 M, pH 1.0) and potassium acetate buffer (0.4 M, pH 4.5). After equilibration for 30 min at room temperature (25 ± 2 °C), absorbance readings were taken at 517 and 700 nm using a UV–Vis spectrophotometer (Bioespectro, spectrophotometer SP220, São Paulo, Brazil). Results were expressed as mg cyanidin-3-O-glucoside equivalents per 100 mL of sample (mg C3G·100 mL^{-1}).

2.3.4. Antioxidant Capacity

Antioxidant capacity was evaluated using the ABTS and DPPH radical scavenging assays, following Re et al. (1999) [19] and Brand-Williams et al. (1995) [20], respectively, with adaptations described by Rufino et al. (2007) [21]. Extracts were obtained using 50% methanol and 70% acetone. For the ABTS assay, the radical solution was prepared from ABTS (7 mmol L^{-1}) and potassium persulfate (140 mmol L^{-1}), with absorbance adjusted to 0.700 ± 0.02 at 734 nm. The reaction time was 6 min. For the DPPH assay, the radical solution was prepared in 95% ethanol (absorbance ≈ 1.1 at 515 nm) and allowed to react with the extracts for 40 min in the dark. Absorbance was measured at 515 nm. Trolox was used as the standard (100–2000 µmol L^{-1}), and results were expressed as µmol Trolox equivalents per gram of sample (µmol TE g^{-1}).

2.4. Stability Study

The concentrated blackberry juice was stored in amber glass bottles under frozen conditions. Samples were analyzed over a storage period of three months for total phenolic content, total monomeric anthocyanins, antioxidant capacity, and color parameters (L*, a*, b*).

2.5. Preparation of Fruit Juice Blends

Five fruit juice blends were designed to evaluate the bioaccessibility of bioactive compounds from blackberry. The formulations were prepared using apple juice, orange juice, and blackberry concentrate, with the apple fraction fixed at 50% (*v*/*v*) in all samples, while the proportions of orange juice and blackberry concentrate were varied to obtain increasing levels of blackberry-derived compounds (Table 1).

Table 1. Fruit juice blend formulations.

Formulations	Apple (%)	Orange (%)	Blackberry (%)
F1	50	50.0	0
F2	50	37.5	12.5
F3	50	25.0	25.0
F4	50	12.5	37.5
F5	50	0	50.0

Formulation F1 (50% apple and 50% orange juice) was used as a control without blackberry addition. Formulations F2 to F5 contained 12.5, 25, 37.5, and 50% blackberry concentrate, respectively, replacing equivalent volumes of orange juice. This substitution scheme resulted in a gradual transition from an apple–orange matrix (F1) to an apple–blackberry matrix (F5). The gradient in blackberry concentration enabled a systematic evaluation of how increasing levels of phenolic compounds and anthocyanins, together with modifications in matrix composition, influenced antioxidant capacity, total phenolic content, color parameters, and the in vitro bioaccessibility of blackberry bioactive compounds.

2.6. In Vitro Gastrointestinal Digestion

Simulated gastrointestinal digestion was performed according to the INFOGEST protocol, using a sequential three-step simulation of the oral, gastric, and intestinal phases [22]. During the oral phase, 5 g of the sample were mixed with simulated salivary fluid (1:1, *w*/*v*) containing amylase (75 U/mL, pH 7.0) and incubated at 37 °C for 2 min. Subsequently, the gastric phase was initiated by the addition of simulated gastric fluid (1:1, *v*/*v*) containing pepsin (2000 U/mL, pH 3.7), followed by incubation at 37 °C for 2 h. For the intestinal phase, simulated intestinal fluid (1:1, *v*/*v*) supplemented with bile salts (6.7 mM) and pancreatin (100 U/mL, pH 7.5) were added, and the mixture was incubated at 37 °C for 2 h. At the end of the digestion process, samples were centrifuged at 4000 rpm for 10 min at 4 °C, and the resulting supernatant was collected as the potentially absorbable fraction. This fraction was used for the determination of vitamin C and anthocyanin contents.

Bioaccessibility was calculated as the ratio between the concentration of each compound in the digested fraction (supernatant) and its initial concentration prior to digestion, with results expressed as a percentage.

2.7. Determination of Anthocyanins by High-Performance Liquid Chromatography (HPLC)

Anthocyanin analysis was performed by high performance liquid chromatography (HPLC), according to the methodology described by Gouvêa et al. (2015) [23]. Chromatographic separation was carried out on a BDS C_{18} Thermo^{TM} column (100 × 4.6 mm; 2.4 µm), maintained at 40 °C. The mobile phase consisted of acetonitrile and aqueous solution of 5% formic acid, operating under gradient elution mode, as described in the reference literature. The injection volume was 20 µL, with a flow rate of 1.0 mL/min and a total chromatographic run time of 20 min. Anthocyanin detection was performed using a UV-Vis detector at 520 nm. Quantification was carried out by external standardiza-

tion using cyanidin-3-O-glucoside as the standard, and results were expressed as mg of cyanidin-3-O-glucoside equivalents per 100 mL of sample.

2.8. Statistical Analysis

All analyses were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical differences among means were evaluated by analysis of variance (ANOVA), followed by Tukey's post hoc test, at a significance level of $\alpha = 0.05$. Statistical analyses were conducted using Statistica software (version 10.0, StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Nanofiltration Process

A decline in permeate flux occurred during the nanofiltration of blackberry juice, decreasing from 6.33 to 1.86 $kg \cdot h^{-1} \cdot m^{-2}$ after 90 min of operation, corresponding to an approximate reduction of 70%. This behavior indicates a progressive increase in filtration resistance, primarily associated with concentration polarization and the formation of a fouling layer on the membrane surface. Such a trend is commonly reported in nanofiltration processes involving phenolic-rich fruit matrices, where the accumulation of solutes near the membrane interface intensifies mass transfer limitations and contributes to flux deterioration [11]. Additionally, the average operating temperature (~40 °C) likely provided a favorable balance between permeate flux and anthocyanin stability, as moderate temperature increases enhance mass transfer while remaining sufficiently low to limit the thermal degradation of heat-sensitive phenolic compounds [9].

Compared with prior studies on membrane concentration of fruit juices, the nanofiltration process demonstrated enhanced enrichment of bioactive compounds. Arend et al. (2017) [24] reported CF values below 1.5 for phenolics and anthocyanins in strawberry juice, whereas this pilot-scale process achieved CF ≈ 2.2 for monomeric anthocyanins and 1.9 for total phenolics. Although Acosta et al. (2017) [25] described high anthocyanin retention in blackberry juice, their analysis focused mainly on solute rejection and permeate behavior. Here, process performance is further linked to concentrate stability, application in blended beverages, and anthocyanin bioaccessibility, highlighting the technological relevance of the approach.

Table 2 presents the physicochemical and bioactive characterization of the fractions obtained during the nanofiltration of blackberry juice using a spiral-wound module. Significant differences ($p < 0.05$) among fractions were observed for most parameters, indicating effective separation and concentration promoted by the membrane process.

The antioxidant capacity measured by ABTS and DPPH followed the same concentration trend as the phenolic compounds across the nanofiltration fractions. The retentate exhibited increases in antioxidant activity, consistent with the increase in total phenolics and anthocyanins. Conversely, the permeate contained significantly lower levels of phenolics and anthocyanins, confirming their selective retention due to molecular size and interactions with the polyamide membrane, which explains the reduced antioxidant capacity observed in this fraction.

This parallel behavior highlights the strong relationship between antioxidant activity and phenolic composition in blackberry juice, supporting previous reports that nanofiltration selectively retains phenolics and anthocyanins, leading to proportional increases in ABTS and DPPH responses [24]. Likewise, higher anthocyanin contents in blackberry fractions have been directly linked to greater antioxidant capacity, reinforcing their role as key contributors to antioxidant activity [5].

Although the permeate exhibited markedly lower phenolic and anthocyanin contents, the absence of detailed compositional profiling limits the identification of individual per-

meating compounds. Nanofiltration membranes with nominal molecular weight cut-off (MWCO) values of approximately 150–300 Da are known to preferentially allow the passage of low-molecular-weight phenolics while retaining larger flavonoids and anthocyanins through steric hindrance and electrostatic interactions [11]. This behavior is consistent with the reduced antioxidant capacity observed in the permeate and aligns with previous reports describing partial transmission of simple phenolic acids alongside strong retention of anthocyanins and flavonols [9].

Table 2. Characterization of the fractions obtained during the nanofiltration process of blackberry juice using a spiral-wound module.

Parameter	Pulp	Feed (Centrifuged Pulp)	Permeate	Retentate	CF
Antioxidant capacity (ABTS) (μmol·mL^{-1})	8.28 ± 0.81 ^{a}	9.31 ± 0.46 ^{a}	1.90 ± 0.11 ^{b}	18.04 ± 0.40 ^{c}	1.9
Antioxidant capacity (DPPH) (μmol·mL^{-1})	4.28 ± 0.08 ^{a}	4.76 ± 0.16 ^{a}	1.48 ± 0.07 ^{b}	8.05 ± 0.36 ^{c}	1.7
Total phenolic content (mg GAE·100 mL^{-1})	143.69 ± 7.33 ^{a}	171.55 ± 4.42 ^{b}	47.15 ± 1.03 ^{c}	326.85 ± 2.97 ^{d}	1.9
Total Monomeric anthocyanins (mg C3G·100 mL^{-1})	26.8 ± 1.93 ^{a}	25.1 ± 0.49 ^{a}	5.8 ± 0.09 ^{b}	54.3 ± 2.67 ^{c}	2.2
pH	3.50 ± 0.01 ^{a}	3.48 ± 0.01 ^{a}	3.51 ± 0.01 ^{a}	3.40 ± 0.02 ^{b}	-
Titratable acidity (g citric acid·100 mL^{-1})	14.05 ± 0.40 ^{a}	13.97 ± 0.22 ^{a}	13.65 ± 1.24 ^{a}	22.13 ± 0.67 ^{b}	1.6
Soluble solids (°Brix)	5.4 ± 0.1 ^{a}	5.5 ± 0.1 ^{a}	3.1 ± 0.0 ^{b}	10.6 ± 0.1 ^{c}	1.9
Total solids (% *w*/*v*)	4.07 ± 0.20 ^{a}	4.35 ± 0.03 ^{a}	2.03 ± 0.07 ^{b}	8.35 ± 0.03 ^{c}	1.9
L*	-	26.47 ± 0.17 ^{c}	27.27 ± 0.14 ^{b}	26.41 ± 0.73 ^{b}	-
a*	-	2.82 ± 0.08 ^{b}	6.23 ± 0.31 ^{a}	4.98 ± 0.17 ^{b}	-
b*	-	0.50 ± 0.04 ^{b}	2.60 ± 0.19 ^{a}	1.78 ± 0.48 ^{b}	-

CF—concentration factor. Values in the same line with different letters are significantly different ($p < 0.05$).

Regarding physicochemical parameters, pH values remained relatively stable among the pulp, feed, and permeate fractions, while a slight but significant decrease was observed in the retentate (pH 3.40 ± 0.02). This behavior is consistent with the concentration of organic acids, as confirmed by the higher titratable acidity observed in the retentate (22.13 ± 0.67 g citric acid 100 mL^{-1}), corresponding to a concentration factor of 1.6.

The concentration effect was also evident for soluble solids and total solids. The retentate showed approximately a twofold increase in soluble solids (10.6 ± 0.1 °Brix) and total solids (8.35 ± 0.03% *m*/*v*) compared to the feed, reflecting the efficient removal of water through the membrane and the retention of solutes with higher molecular weight. In contrast, the permeate presented significantly lower values for these parameters, indicating effective fractionation of the juice components.

Overall, the results demonstrate that nanofiltration using a spiral-wound module is an efficient strategy for concentrating bioactive compounds, particularly phenolics and anthocyanins, in blackberry juice while maintaining physicochemical stability. The concentrated juice presents enhanced antioxidant capacity and compositional characteristics suitable for application as a functional ingredient or for the development of value-added beverages. Studies by Castro-Muñoz (2024) [26] have demonstrated the high efficiency of nanofiltration in retaining phenolic fractions, with recoveries exceeding 97%, while preserving thermolabile compounds due to operation at mild temperatures.

Color is one of the main quality parameters in the food industry, as it directly influences consumer perception and acceptance. In addition, color attributes may indicate the presence and concentration of anthocyanins and flavonoids in food matrices [24]. The colorimetric behavior observed among the different streams of the spiral-wound module highlights the selectivity of the system toward pigmented fractions of the matrix (Table 2). The fractions resulting from the concentration of blackberry juice by nanofiltration are shown in Figure 3.

Figure 3. Fractions of the blackberry juice concentration process by nanofiltration.

The increase in L, a*, and b* values in the permeate indicate the passage of low-intensity chromophoric compounds, producing a clearer solution with a slight shift toward red–yellow hues. This effect is likely related to the permeation of low-molecular-weight phenolics and anthocyanin degradation products, such as phenolic acids and flavonoids, which contribute weakly to color [27]. Similar patterns have been reported for pigmented matrices subjected to membrane filtration, where size- and structure-based selectivity promotes pigment enrichment in the retentate and clarification of the permeate, as observed in the nanofiltration of red wines [13].

3.2. Storage Stability

Table 3 and Figure 4 present the evolution of bioactive compounds, antioxidant capacity, and physicochemical parameters of the concentrated blackberry juice throughout the storage period under freezing conditions.

Table 3. Bioactive compounds and antioxidant capacity of nanofiltered blackberry juice at different storage times under freezing conditions.

Parameter	Storage Time (Days)				
	0	15	30	60	90
Total Monomeric Anthocyanins (mg C3G·100 mL^{-1})	106.03 ± 1.10 ^{a}	105.98 ± 3.74 ^{a}	107.18 ± 2.32 ^{a}	102.67 ± 2.17 ^{a}	96.13 ± 2.28 ^{b}
Total Phenolic Content (mg AGE·100 mL^{-1})	398.23 ± 3.52 ^{a}	403.01 ± 3.12 ^{a}	373.66 ± 2.12 ^{ab}	344.25 ± 2.48 ^{bc}	329.07 ± 5.70 ^{c}
Antioxidant Capacity (ABTS) µmol·mL^{-1}	27.68 ± 2.13 ^{b}	36.44 ± 1.66 ^{a}	32.14 ± 2.94 ^{ab}	29.93 ± 1.21 ^{b}	28.96 ± 1.21 ^{b}
Antioxidant Capacity (DPPH) µmol·mL^{-1}	19.40 ± 1.60 ^{ab}	20.22 ± 0.20 ^{b}	19.45 ± 0.05 ^{ab}	16.70 ± 0.66 ^{bc}	14.82 ± 1.06 ^{c}
pH	3.19 ± 0.01 ^{bc}	3.26 ± 0.00 ^{a}	3.19 ± 0.01 ^{c}	3.25 ± 0.02 ^{a}	3.24 ± 0.02 ^{ab}
Total titratable acidity (g citric acid·100 mL^{-1})	21.29 ± 0.16 ^{a}	20.77 ± 1.90 ^{a}	21.38 ± 0.06 ^{a}	21.39 ± 0.68 ^{a}	21.34 ± 0.67 ^{a}
Soluble solids (°Brix)	15.2 ± 0.05 ^{a}	14.8 ± 0.05 ^{ab}	14.4 ± 0.25 ^{bc}	14.3 ± 0.15 ^{c}	14.0 ± 0.05 ^{c}
Total solids (% *m*/*v*)	13.60 ± 0.13 ^{a}	14.19 ± 0.09 ^{a}	13.34 ± 0.06 ^{a}	12.76 ± 3.13 ^{a}	12.19 ± 2.62 ^{a}
L*	29.40 ± 0.06 ^{b}	29.51 ± 0.06 ^{b}	27.83 ± 0.18 ^{c}	27.69 ± 0.27 ^{c}	30.90 ± 0.43 ^{a}
a*	8.10 ± 0.03 ^{b}	7.33 ± 0.01 ^{c}	8.28 ± 0.09 ^{a}	7.46 ± 0.06 ^{a}	8.20 ± 0.08 ^{a}
b*	1.99 ± 0.07 ^{b}	1.21 ± 0.03 ^{a}	3.09 ± 0.18 ^{a}	3.05 ± 0.20 ^{a}	1.29 ± 0.09 ^{c}

Different letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

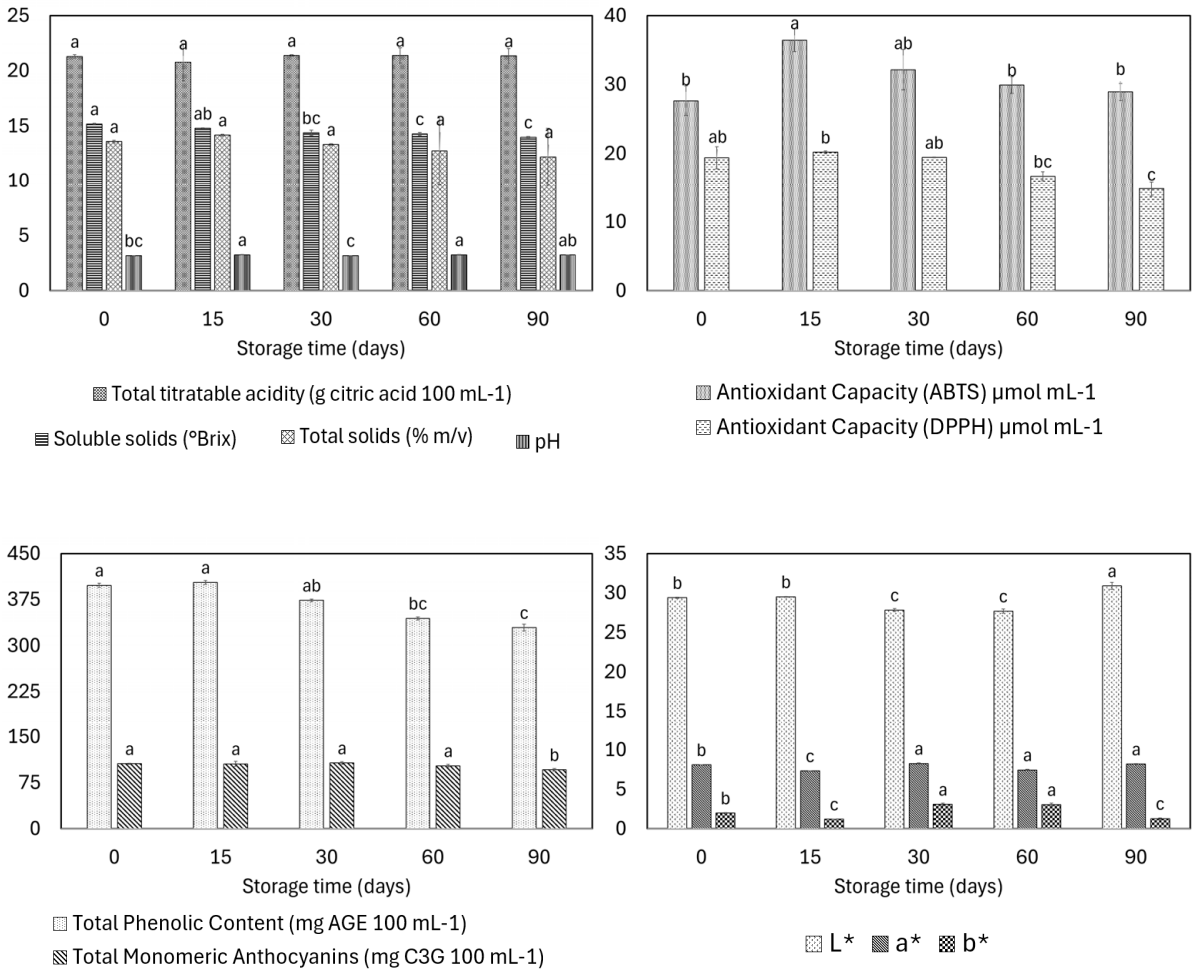

Figure 4. Evolution of physicochemical characteristics, bioactive compounds, antioxidant capacity, and color during storage. Different letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

Overall, these results corroborate the literature regarding the superiority of frozen storage in maintaining the chemical quality of concentrated blackberry juices, especially with respect to the preservation of phenolic compounds. After 90 days of storage, monomeric anthocyanin levels remained largely preserved, with only a slight decrease observed at the end of the storage period. This stability may be attributed to the inhibition of enzymatic and oxidative degradation reactions at very low temperatures. Patras et al. (2010) [28] similarly reported that freezing is among the most effective preservation methods for anthocyanins in food matrices. Acosta et al. (2017) [25] also applied nanofiltration membranes with a molecular weight cut-off of approximately 200 Da to blackberry juice, achieving significant concentration of anthocyanins and ellagitannins, as well as satisfactory stability during frozen storage.

Although total phenolic content gradually decreased over time, values remained high throughout storage, reflecting the elevated concentration achieved by nanofiltration and frozen preservation. The slight fluctuations in L and b* values over the 90-day storage period likely reflect the gradual chemical evolution of compounds in the nanofiltered concentrate. Even under freezing conditions, slow degradation processes such as structural rearrangements and residual oxidation may occur [29], alongside condensation reactions between anthocyanins and other phenolics that promote polymeric pigment formation. These structural changes alter light absorption and scattering, leading to subtle darkening and hue shifts while contributing to color stabilization. Variations in total phenolics and

antioxidant capacity may additionally be linked to the higher oxidation susceptibility of low-molecular-weight phenolics and the transient formation of intermediate compounds with enhanced radical scavenging activity [29]. Consistent with reports for frozen and concentrated berry matrices, these changes suggest underlying chemical dynamics rather than instrumental variability. The higher a* and b* values observed in the retentate further indicate the preferential retention of intact anthocyanins and copigmented complexes during concentration, supporting the overall physicochemical stability of the concentrate throughout storage.

Similarly, Cai et al. (2021) [29] reported that blueberry (*Vaccinium* spp.) juice concentrates obtained by nanofiltration and related membrane technologies exhibited improved functional stability during storage at 4 °C, indicating that low storage temperature plays a key role in preserving phenolic compounds in concentrated systems.

Color stability is closely associated with anthocyanin preservation, as these pigments are primarily responsible for the red–purple coloration of blackberry juice. The relatively stable L* values observed during storage indicate limited clarification or pigment loss, which is consistent with the minor changes observed in monomeric anthocyanin content. Slight fluctuations in L* over time may be related to pigment rearrangements or copigmentation effects rather than extensive degradation (Table 3).

The a* parameter, which represents red color intensity and is directly linked to anthocyanin concentration, remained relatively stable throughout the storage period, supporting the observed preservation of anthocyanins under frozen conditions. Variations in b* values may reflect changes in copigments or the formation of minor anthocyanin degradation products, which can subtly shift color toward yellow–red hues without significantly affecting total monomeric anthocyanin content.

Frozen storage effectively preserved not only the anthocyanin content but also the visual quality of the nanofiltered blackberry juice. The strong correspondence between anthocyanin stability and color parameters reinforces the suitability of combining nanofiltration and frozen storage to obtain stable, visually appealing, and functionally enriched fruit juice products.

3.3. Characterization of the Juice Blends

Table 4 summarizes the antioxidant capacity, total phenolic content, monomeric anthocyanins, and color parameters of the different juice blend formulations, highlighting significant differences among the samples.

Table 4. Characterization of fruit juice blends containing apple, orange, and blackberry concentrate.

Parameter	F1	F2	F3	F4	F5
Antioxidant Capacity (TEAC) (μmol Trolox/mL)	0.39 ± 0.05 ^{e}	2.32 ± 0.14 ^{d}	4.45 ± 0.19 ^{c}	6.95 ± 0.58 ^{b}	8.04 ± 0.29 ^{a}
Antioxidant Capacity (DPPH) μmol/mL	0.42 ± 0.02 ^{e}	1.07 ± 0.08 ^{d}	1.99 ± 0.06 ^{c}	3.06 ± 0.03 ^{b}	3.16 ± 0.19 ^{a}
Total Phenolic Content (mg AGE·100 mL^{-1})	46.06 ± 0.44 ^{e}	103.38 ± 0.75 ^{d}	140.79 ± 3.23 ^{c}	176.21 ± 0.63 ^{b}	245.09 ± 3.51 ^{a}
Total Monomeric Anthocyanins (mg C3G·100 mL^{-1})	-	10.37 ± 0.50 ^{d}	18.27 ± 1.33 ^{c}	37.37 ± 2.79 ^{b}	42.20 ± 3.07 ^{a}
L*	45.07 ± 0.07 ^{a}	31.37 ± 0.20 ^{b}	29.49 ± 0.37 ^{c}	28.13 ± 0.30 ^{d}	27.69 ± 0.27 ^{d}
a*	0.36 ± 0.05 ^{c}	9.77 ± 0.06 ^{a}	9.01 ± 0.04 ^{a}	7.70 ± 0.21 ^{b}	7.46 ± 0.06 ^{b}
b*	14.24 ± 0.25 ^{a}	2.27 ± 0.11 ^{d}	2.66 ± 0.27 ^{c}	3.20 ± 0.34 ^{b}	3.05 ± 0.20 ^{b}

F1—50% apple, 50% orange, and 0% blackberry; F2—50% apple, 37.5% orange, and 12.5% blackberry; F3—50% apple, 25% orange, and 25% blackberry; F4—50% apple, 12.5% orange, and 37.5% blackberry; F5—50% apple, 0% orange, and 50% blackberry. Different letters within the same line indicate significant differences according to Tukey's test ($p < 0.05$).

A clear and statistically significant effect of increasing blackberry concentrate on the bioactive composition, antioxidant capacity, and color attributes of the juice blends was observed (Table 4). As the proportion of blackberry increased from F1 to F5, a progressive rise in TEAC, DPPH, total phenolic content, and total monomeric anthocyanins was observed, confirming blackberry as the main contributor to the functional properties of the formulations. The control formulation (F1), without blackberry, exhibited the lowest antioxidant and phenolic values, whereas F5 (50% blackberry concentrate) showed the highest levels, with significant differences among all formulations ($p < 0.05$). These compositional changes were directly reflected in the color parameters. A significant decrease in lightness (L*) accompanied by an increase in redness (a*) was observed with increasing blackberry content, indicating the intensification of anthocyanin-derived pigmentation.

In contrast, the reduction in b* values reflects the progressive replacement of the yellow carotenoid-rich orange juice by the anthocyanin-rich blackberry concentrate. The negative a* value and high L* and b* observed for F1 are consistent with the absence of anthocyanins and the predominance of citrus pigments. Moreover, the similar color values observed for F4 and F5, despite further increases in bioactive compounds, suggest a saturation effect, in which additional anthocyanins do not result in proportional visual color changes. Overall, these results highlight the strong relationship between anthocyanin content, antioxidant capacity, and color modulation, demonstrating that blackberry concentrate is an effective functional and natural ingredient for enhancing both the nutritional and visual quality of the fruit juice blends.

A progressive increase in the concentrations of cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and unidentified anthocyanins was observed from formulations F2 to F5 (Table 5). This behavior is consistent with recent reviews describing blackberry as one of the main dietary sources of anthocyanins, with cyanidin-3-O-glucoside being the predominant anthocyanin in fruits of the *Rubus* genus and the primary contributor to the antioxidant activity associated with these products [30].

Table 5. Anthocyanin profile identified in the juice blends from the different formulations.

Anthocyanins	F2	F3	F4	F5
Cyanidin-3-O-glucoside (mg·100 mL^{-1})	1.36 ± 0.29 ^{d}	2.66 ± 0.01 ^{c}	10.22 ± 0.33 ^{b}	15.34 ± 0.93 ^{a}
Cyanidin-3-O-rutinoside (mg·100 mL^{-1})	0.11 ± 0.02 ^{d}	0.17 ± 0.01 ^{c}	0.85 ± 0.01 ^{b}	1.29 ± 0.03 ^{a}
Unidentified anthocyanin (mg·100 mL^{-1})	0.06 ± 0.01 ^{d}	0.08 ± 0.00 ^{c}	0.38 ± 0.03 ^{b}	0.47 ± 0.01 ^{a}
Unidentified anthocyanin (mg·100 mL^{-1})	0.05 ± 0.01 ^{d}	0.08 ± 0.00 ^{c}	0.40 ± 0.03 ^{b}	0.54 ± 0.01 ^{a}
Sum of monomeric anthocyanins (mg·100 mL^{-1})	1.56 ± 0.33 ^{d}	2.99 ± 0.01 ^{c}	11.84 ± 0.28 ^{b}	17.63 ± 0.94 ^{a}

F2—50% apple, 37.5% orange, and 12.5% blackberry; F3—50% apple, 25% orange, and 25% blackberry; F4—50% apple, 12.5% orange, and 37.5% blackberry; F5—50% apple, 0% orange, and 50% blackberry. Different letters within the same line indicate significant differences according to Tukey's test ($p < 0.05$).

The significant increase in total monomeric anthocyanins and antioxidant capacity with increasing blackberry proportion confirms that the gradual substitution of orange juice with blackberry juice promotes functional enrichment of the juice blend. Recent studies on red fruit juices and extracts have shown that formulations with higher blackberry content exhibit greater anthocyanin diversity and concentration, resulting in intensified coloration and enhanced antioxidant potential [31].

The presence of unidentified anthocyanins is frequently reported in complex *Rubus* spp.-based matrices, as these fruits contain a wide range of minor anthocyanins, including

acylated derivatives, less common glycosides, and copigmentation products, which may not be fully resolved or identified when specific commercial standards are not available [18].

3.4. In Vitro Gastrointestinal Digestion of the Juice Blend

The bioaccessibility of cyanidin-3-O-glucoside varied among the evaluated formulations, highlighting the combined influence of initial concentration and food matrix composition on the fraction released after simulated in vitro gastrointestinal digestion. Table 6 shows the bioaccessibility of cyanidin-3-O-glucoside in the juice blends. Formulation F2 showed values below the limit of quantification, whereas formulations F3, F4, and F5 exhibited measurable concentrations in the digested fraction.

Table 6. Bioaccessibility of cyanidin-3-O-glucoside in the fruit juice blends.

Formulation	Cyanidin-3-O-glucoside	
	Digested Fraction (mg·100 mL)	**Bioaccessibility (%)**
F2	NQ	-
F3	0.57 ± 0.05^{c}	18.93 ± 4.27^{ab}
F4	1.43 ± 0.14^{b}	14.67 ± 1.50^{b}
F5	3.52 ± 0.01^{a}	22.98 ± 0.08^{a}

F2—50% apple, 37.5% orange, and 12.5% blackberry; F3—50% apple, 25% orange, and 25% blackberry; F4—50% apple, 12.5% orange, and 37.5% blackberry; F5—50% apple, 0% orange, and 50% blackberry. NQ = Not quantified. Different letters within the same column indicate significant differences according to Tukey's test ($p < 0.05$).

For cyanidin-3-O-glucoside, concentrations in the digested fraction ranged from 0.57 to 3.52 mg·100 mL^{-1}, corresponding to bioaccessibility values between 14.67% and 22.98%. The reduction observed after digestion is consistent with previous studies demonstrating that cyanidin-3-O-glucoside in liquid systems undergoes substantial losses during the simulated intestinal phase. These losses are primarily attributed to structural instability under near-neutral pH, where hydration of the flavylium cation leads to less stable hemiketal and chalcone forms, compromising anthocyanin stability and solubility, as previously reported for red wine and other polyphenol-rich matrices [28,32].

Additionally, interactions with matrix components such as carbohydrates, proteins, lipids, and polysaccharides may promote complex formation or binding to macromolecules, limiting anthocyanin release and increasing susceptibility to degradation during digestion [33]. Similar behavior has been reported for other complex liquid matrices, such as red wines, in which significant anthocyanin losses are observed after simulated gastrointestinal digestion [32]. In fruit-based beverages, anthocyanin stability is strongly influenced by matrix composition, ingredient proportions, and physicochemical interactions, which can modulate both the initial release and the progressive degradation of these compounds throughout the digestive process [27].

The formulations F3, F4, and F5 showed that increasing the blackberry proportion did not lead to a linear improvement in cyanidin-3-O-glucoside bioaccessibility, underscoring the influence of matrix-related factors over anthocyanin concentration alone. Despite its higher blackberry content, F4 exhibited the lowest bioaccessibility, likely due to stronger interactions between anthocyanins and matrix components such as pectins and dietary fibers, which may have limited their release during digestion [33,34].

The non-proportional relationship between anthocyanin content and bioaccessibility reflects a pronounced matrix effect. Blackberry polyphenols may interact with sugars, organic acids, and dietary fibers from the apple–orange base, forming reversible complexes or becoming physically entrapped within the matrix, which can limit anthocyanin release, solubilization, and stability during gastrointestinal digestion [28]. Consequently,

higher anthocyanin concentrations did not yield proportionally greater bioaccessible fractions, indicating that bioaccessibility depends not only on initial content but also on the physicochemical properties of the surrounding matrix.

In studies evaluating different fruit juices, including commercial blackberry juice and black grape juice, Mihaylova et al. (2021) [35] reported pronounced reductions in anthocyanin bioaccessibility after in vitro gastrointestinal digestion, indicating that in liquid matrices rich in these pigments, stability during the intestinal phase is limited. These reductions were primarily attributed to the intrinsic structural instability of anthocyanins under near-neutral pH conditions, with anthocyanins being more susceptible to degradation than other classes of phytochemicals, such as phenolic acids and non-anthocyanin flavonoids. However, reduced intestinal bioaccessibility should not be interpreted solely as a result of degradation. Evidence indicates that part of the anthocyanins may be absorbed in the stomach, even under acidic conditions, reducing the fraction available for intestinal recovery. In vitro digestion studies combined with gastric and intestinal transport models have demonstrated significant gastric absorption of anthocyanins from dark-colored fruits such as jabuticaba, jamelão, and jambo, suggesting that early absorption contributes to the low intestinal bioaccessibility of these compounds [36].

Furthermore, even in relatively homogeneous liquid matrices, anthocyanin bioaccessibility does not depend exclusively on the initial concentration of the compounds but is strongly modulated by interactions with matrix components such as sugars, organic acids, and other phenolic compounds. These interactions may influence both the solubilization and the transient stability of anthocyanins during gastrointestinal digestion [35]. Despite the overall reduction observed after digestion, the persistence of a measurable bioaccessible fraction indicates that matrix interactions may partially protect anthocyanins from complete degradation, delaying their transformation under intestinal conditions.

It is also important to consider that anthocyanins not released in the small intestine may reach the colon, where they can be extensively metabolized by gut microbiota into low-molecular-weight phenolic compounds with higher absorption potential and biological activity. Therefore, limited intestinal bioaccessibility does not preclude the biological relevance of anthocyanin-rich matrices [37].

Studies employing the INFOGEST protocol consistently demonstrate that the intestinal phase represents the main barrier to anthocyanin stability, regardless of the food matrix evaluated. Sánchez-Velázquez et al. (2021) [38] when investigating wild and commercial blackberries, observed that although part of the anthocyanins is released and partially preserved during the oral and gastric phases, marked degradation occurs during the intestinal stage, with losses exceeding 70%. These losses are mainly attributed to the increase in pH and the consequent structural instability of anthocyanins, indicating that chemical structure and pH are the primary determinants of anthocyanin fate during digestion.

4. Conclusions

The application of nanofiltration using a spiral-wound polyamide membrane proved to be a highly effective non-thermal strategy for concentrating blackberry juice. The process allowed for a significant enrichment of bioactive compounds, achieving concentration factors of 1.9 for total phenolics and 2.2 for total monomeric anthocyanins while maintaining the physicochemical stability of the matrix.

The stability study demonstrated that frozen storage is an ideal method for preserving the quality of the concentrate. Over 90 days, the total monomeric anthocyanin content and antioxidant capacity remained largely preserved, with color parameters (L*, a*, b*) showing only minor variations, ensuring the maintenance of the product's visual appeal.

Furthermore, the incorporation of the blackberry concentrate into fruit juice blends successfully enhanced their functional profile. The progressive increase in blackberry concentration resulted in a linear rise in antioxidant potential and intensified the characteristic red pigmentation of the beverages. Although the bioaccessibility of anthocyanins may be challenged by the gastrointestinal environment, the use of nanofiltration provides a potent ingredient for the development of value-added functional beverages.

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