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Bioactive and Chemical Characterization of Grape Pomace from Different Grape Varieties for Its Valorization as a Functional Ingredient in Gummy Candies

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Abstract

Grape pomace (GP), a major wine industry residue, contains substantial amounts of phenolic bioactive compounds whose valorization potential remains underexploited. In this study, pomaces from the Syrah, Malbec, Cabernet Sauvignon, Tempranillo, Nebbiolo-Sangiovese (blend), Cabernet Franc, Merlot, and Ensamble varieties were characterized in terms of total phenolic content (TPC), flavonoids, anthocyanins, tannins, and antioxidant capacity. Grape pomace flour (GPF) was obtained by drying, milling, and sieving (<250 µm) GP at 60 °C, and its functional properties were compared with GP and two commercial pomace flours (H1 and H2). The GPFs had a TPC of 3.48–12.06 mg gallic acid equivalents g^{-1} dry basis (d.b.), flavonoid content of 11.78–71.65 mg quercetin equivalents g^{-1} d.b., anthocyanin content of 41.46–291.85 mg cyanidin-3-glucoside equivalents g^{-1} d.b., tannin content of 18.85–45.98 mg (+)-catechin equivalents g^{-1} d.b., and antioxidant capacity of 304.2–768.3 µmol Trolox equivalents g^{-1} d.b. The Malbec variety exhibited the best bioactive profile and was incorporated into a gelatin-based gummy matrix. Color and bioactive compound stability were measured over 30 days of storage at 40 °C and compared with a commercial flour (H1). The valorization of GP through drying and milling is a viable strategy for obtaining a flour with potential as a functional ingredient due to its bioactive composition and as a natural colorant in the food industry.

Keywords: bioactive compounds; phenolic compounds; anthocyanins; antioxidant capacity; winery by-products; confectionery products

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1. Introduction

The wine industry produced 258 million hectoliters of wine worldwide in 2022, making it one of the most important agroindustries globally. However, this sector is also responsible for generating at least 200 kg of waste for every 750 L of wine [1,2]. There is widespread concern about the potential environmental impact of these residues, particularly as a result of their high phenolic content and the potential acidification of soil and water at disposal sites [3], as well as their high organic matter load, which can attract pests—such as insects, rodents, and birds—posing a sanitary risk to nearby populations [1].

Winery waste is composed of grape pomace (GP), stems, lees, branches, leaves, and unprocessed grapes. GP is the main residue generated, representing up to 60% of the total winemaking waste. GP consists of skins (~50%), seeds (~25%), and stems (~25%) [4]. It is typically composed of water (50–60%), dietary fiber (20–30%), carbohydrates (5–15%), protein (8–15%), lipids (3–7%), polyphenols (5–10%), and ash (3–5%) [5]. About 70% of the bioactive compounds in wine grapes remain in the GP, since only ~30% is transferred from the grape to the wine [6]. As a result, GP is a rich source of phenolic acids (~17.46–47.27 mg gallic acid equivalents g^{-1} d.b.), resveratrol (~4.58 μg g^{-1} d.b.), anthocyanins (~0.8–48.3 mg cyanidin-3-glucoside equivalents g^{-1} d.b.), and flavonoids (~11.3 mg catechin equivalents g^{-1} d.b.) [7–9]. However, its chemical composition is strongly influenced by the grape cultivar, environmental conditions, berry ripeness, and winemaking process [9,10].

In general, the bioactive compounds contained in GP stand out for their antioxidant, anti-inflammatory, and other biological activities, which have been demonstrated in in vitro and in vivo studies and are associated with the prevention of diseases, including certain types of cancer, gastrointestinal disorders such as irritable bowel syndrome, and diseases related to oxidative stress [11]. Furthermore, these functions could be enhanced by the high dietary fiber content of GP, which contributes to gut health maintenance through modulation of the microbiota and production of beneficial metabolites [12]. In this regard, the potential of GP as a functional ingredient for the development of nutraceutical, cosmetic, and functional food products has been widely studied [10,13], including its use in bakery products, cereal bars and snacks, yogurts and other fermented dairy products, meats and meat analog products, smoothies, and healthy beverages [14,15]. However, research on the incorporation of GP into gummy candies is scarce, and most available studies focused on a single grape variety or a very limited number of them, making it difficult to determine whether the reported functional properties are cultivar-dependent or a general trend.

In addition, it is important to highlight that the utilization of GP as a functional ingredient requires preserving its bioactive compounds during residue conditioning. Managing GP throughout its valorization process involves a series of challenges, as it tends to have high moisture and sugar contents, making it susceptible to enzymatic and microbial degradation [16]. Microbial deterioration has been reported to lead to a loss of between 40 and 87% of the antioxidant capacity and between 70 and 90% of the total phenolic content [17]. In this regard, stabilization of this residue through drying could become a critical step, as it reduces microbial activity, improves the handleability of large volumes of GP, and partially preserves its bioactive compounds, facilitating its transformation into value-added products [18]. However, although drying constitutes an indispensable step for GP valorization, information on the effects of this process on bioactive compound retention across different grape varieties remains scarce.

In this context, the transformation of stabilized GP through drying into a flour rich in fiber and bioactive compounds, and its subsequent incorporation as a functional ingredient in gummy candies, represents a strategy that fits within the circular economy and comprehensive biomass valorization framework. Considering the above, the objectives of this research were as follows: (a) to evaluate the content of bioactive compounds present in GP from different grape varieties after drying, and (b) to evaluate the feasibility of incorporating GP flour into a gelatin-based gummy matrix by measuring the remaining bioactive compounds in this matrix. This work constitutes a starting point for the identification and development of methodologies that allow for the preservation and effective utilization of winemaking residues within a circular economy approach.

2. Materials and Methods

2.1. Grape Pomace Sample Collection

Grape pomace (GP) was donated by a winery located in San Miguel de Allende, Guanajuato State (21°05′06.5″ N latitude and 100°48′03.4″ W longitude), and was collected between July and August 2023. Each variety underwent its own winery-specific vinification process (maceration, fermentation, and pressing), as is standard commercial practice for each varietal profile; consequently, the pomace evaluated reflects real industrial by-product streams rather than standardized experimental material. The varieties were Syrah, Malbec, Cabernet Sauvignon, Tempranillo, Nebbiolo-Sangiovese (a blend of these two varieties in proportions not disclosed by the vineyard), Cabernet Franc, Merlot, and Ensamble (a blend of Cabernet Sauvignon, Merlot, Cabernet Franc, and Tempranillo in proportions not disclosed by the vineyard due to proprietary reasons). All grape varieties were cultivated and processed within the same vineyard, at the winery located within the same production site, and during the same harvest season. All samples were vacuum-packed in Food Saver® brand polyethylene and nylon bags and stored at −20 °C until use.

2.2. Grape Pomace Flour (GPF) Preparation

GP from each variety was subjected to a drying process in a tray oven (MainStays, MG10BFK-B, Foshan, China), arranged in uniform 1 cm thick layers to promote homogeneous heat transfer, with the temperature controlled at 60 °C for 24 h. After drying, the GP samples were allowed to cool to room temperature in a desiccator. Subsequently, the dried GP samples were milled using a blade mill (Mr. Coffee, IDS57-4, Boca Raton, FL, USA) until a homogeneous powder was obtained, which was sieved through a No. 60 mesh (250 µm) to standardize the particle size. Finally, the resulting GP powder was stored at 4 °C in airtight polyethylene containers until use in subsequent analyses. From this point on, the dried, milled, and sieved GP was referred to as grape pomace flour (GPF). Commercial grape pomace flours from the Cabernet Sauvignon (H1) and Merlot (H2) varieties, were obtained from a local market. These commercial flours were included as reference materials, representing products already available on the market, in order to compare the bioactive compound content and technological performance of the GPFs obtained in this study with those of an existing commercial alternative.

2.3. Bioactive Compound Extraction

A 0.25 g sample from each GPF was taken, 5 mL of a methanol–water (50:50 v/v) mixture was added, and the mixture was stirred in the dark for 24 h at 20 ± 2 °C (20 mL of solvent per gram of material). For fresh samples (moisture content of 67.44–74.04%), 1 g of sample from each GP was weighed and ground in a mortar until a paste was obtained, and then the same extraction procedure used for the dried samples was repeated (except that 15.36–19.26 mL of solvent per gram of material was used). After extraction, dissolved solids were removed by centrifugation for 20 min at 1010× g (Velocity 14R refrigerated centrifuge, Dynamica Scientific, Livingston, UK).

2.4. Analysis of Grape Pomace Bioactive Compounds

Determination of total phenolic content (TPC), flavonoids, monomeric anthocyanins, tannins, and antioxidant capacity was carried out using the methods described by Waterhouse et al. [19], Zhishen et al. [20], Taghavi et al. [21], Deshpande and Cheryan [22], and Re et al. [23], respectively, which are described in detail in Appendix A.1. All results were expressed on a dry basis, accounting for the moisture content.

2.5. Grape Pomace Flour Proximate Analysis

The proximate composition of the grape pomace (moisture, crude fat, crude protein, crude fiber, and ash) was determined according to the methods reported in the Mexican standards: NMX-F-428-1982 [24], NOM-F-89-S-1978 [25], NMX-F-608-NORMEX-2011 [26], NMX-F-613-NORMEX-2017 [27], and NMX-F-066-S-1978 [28], respectively. The remaining content was recorded as the carbohydrate content. The results were expressed as a percentage.

2.6. Incorporation and Evaluation of Grape Pomace Flour in a Food Matrix

A commercial pomace flour (H1) and the GPF from our study with the most favorable bioactive compound content (Malbec GPF) were selected for integration into a gummy candy formulation. Color, total phenolic, and anthocyanin content stability in the resulting gummies were evaluated, along with the corresponding kinetic degradation parameters.

2.6.1. Gelatin-Based Gummy Candy Formulation

The gummy samples were formulated according to the method of Rodríguez-Sánchez et al. [29] with some modifications. Each formulation consisted of 5 g of GPF, 25 g of gelatin, 25 g of sucrose, and 0.1 g of citric acid, with water added to reach a final weight of 100 g. The samples were divided into 25 g portions with a thickness of 5 mm and packed in resealable polyethylene bags and stored at 4 °C until the start of the stability assay. A blank was prepared excluding the GPF mass and proportionally adjusting the concentration of gelatin, sucrose, and citric acid to the water volume. Each formulation (Malbec GPF and H1) corresponded to a single batch, from which $n = 3$ sub-samples were taken at each storage time point for the assays described in Sections 2.6.2 and 2.6.3.

2.6.2. Color Stability Evaluation

The color of the gummies was determined using the parameters of the CIELAB color space (L^*, a^*, and b^*) and a Color Flex EZ 45/0 colorimeter (CFEZ1005; Hunter Lab, Reston, VA, USA), with excluded specular directional geometry at 45/0°, and reflectance measurements performed under D65 illumination, at a 10° observation angle against a white background. Color stability was evaluated over 30 days of storage (at 0, 1, 2, 3, 4, 7, 10, 15, 20, 25, and 30 days) at 40 °C; this temperature was selected to accelerate bioactive compound degradation for kinetic modeling purposes. The total color difference (ΔE) was calculated using Equation (1).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* represent the difference in the color parameters between the sample at the beginning of the treatment (day 0; color parameters L^*_0, a^*_0, and b^*_0) and the tested time point (color parameters L^*, a^*, and b^*).

2.6.3. Bioactive Compound Stability Evaluation

To evaluate the stability of total phenolics and anthocyanins in gummies formulated with GPF, the methodology used by Rodríguez-Sánchez et al. [29] was followed, with some modifications. Gummy samples were stored at 40 °C in the absence of light, and samples were taken at 0, 1, 2, 3, 4, 7, 10, 15, 20, 25, and 30 days. For extraction of bioactive compounds, a 1 g sample was taken, 2 mL of a methanol–water solution (50:50 v/v) was added, the mixture was held in a closed container for 30 min at 60 °C, vortexed for 1 min, and then subsequently centrifuged at 1010× g for 10 min. The supernatant was used to determine the total phenolic and anthocyanin concentrations, as well as to quantify the antioxidant capacity.

The degradation rate constant and half-life for total phenolic content and anthocyanins were calculated using a first-order kinetic model (Equations (2) and (3)). To evaluate the suitability of this model, zero-order (Equations (4) and (5)) and second-order (Equations (6) and (7)) kinetic models were additionally fitted to the same data.

$$Ln\left(\frac{C_t}{C_0}\right) = -kt \quad (2)$$

$$t_{1/2} = \frac{Ln(2)}{k} \quad (3)$$

$$C_t = C_0 - kt \quad (4)$$

$$t_{1/2} = \frac{C_0}{2k} \quad (5)$$

$$\frac{1}{C_t} = \frac{1}{C_0} + kt \quad (6)$$

$$t_{1/2} = \frac{1}{(k * C_0)} \quad (7)$$

where C_0 is the concentration of the compound at time 0, C_t is the concentration of the compound at time t, k is the degradation constant, and $t_{1/2}$ is the half-life.

2.7. Statistical Analysis

Grape pomace from each variety was collected as a single composite sample. Results were expressed as the mean ± standard deviation of $n = 3$ analytical replicates obtained from this composite sample. Data were evaluated using a one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$. Tukey's test was applied for mean comparisons. To further test whether the effect of drying on bioactive compound content depended on the cultivar, a two-way ANOVA (cultivar × processing state, including their interaction) was additionally performed on the eight grape varieties, for which both fresh GP and GPF were characterized; the two commercial flours (H1 and H2) were excluded from this analysis since we did not have a fresh counterpart for them. Statistical comparisons are interpreted as exploratory; they do not estimate biological variability (between harvest seasons or between vineyards).

Additionally, principal component analysis (PCA) was performed on cultivar means ($n = 8$), computed from the analytical replicates, as an exploratory tool to identify patterns in the bioactive compound profile of the grape pomace samples. Statistical analyses were performed using MINITAB® V 22.1 software.

3. Results and Discussion

3.1. Bioactive Compound Analysis

GP from the different grape varieties was subjected to a drying process at 60 °C for 24 h to obtain GPF, and the bioactive compound concentrations in the GP and GPF were measured (Figure 1). Overall, the GPF samples showed higher TPCs (3.48–12.06) than fresh GP (2.41–10.59) (Figure 1a). Notably, GPF from the Malbec variety had the highest TPC (12.06 mg GAE g^{-1} d.b.) and flavonoid content (63.37 mg quercetin eq. g^{-1} d.b.), followed by Nebbiolo-Sangiovese GPF (11.79 mg GAE g^{-1} d.b. and 71.65 mg quercetin eq. g^{-1} d.b.); the Syrah, Tempranillo, and Merlot GPFs showed lower values ($p < 0.05$; Figure 1a, b). A two-way ANOVA (cultivar × processing state; Table A1, Appendix A.2) detected a cultivar × processing interaction for both TPC ($F_{7,32} = 70.0$, $p < 0.001$) and flavonoid content ($F_{7,32} = 73.73$, $p < 0.001$), indicating that the difference between the

fresh and dried composite samples varied among the samples evaluated, rather than a generalizable cultivar-specific response to drying.

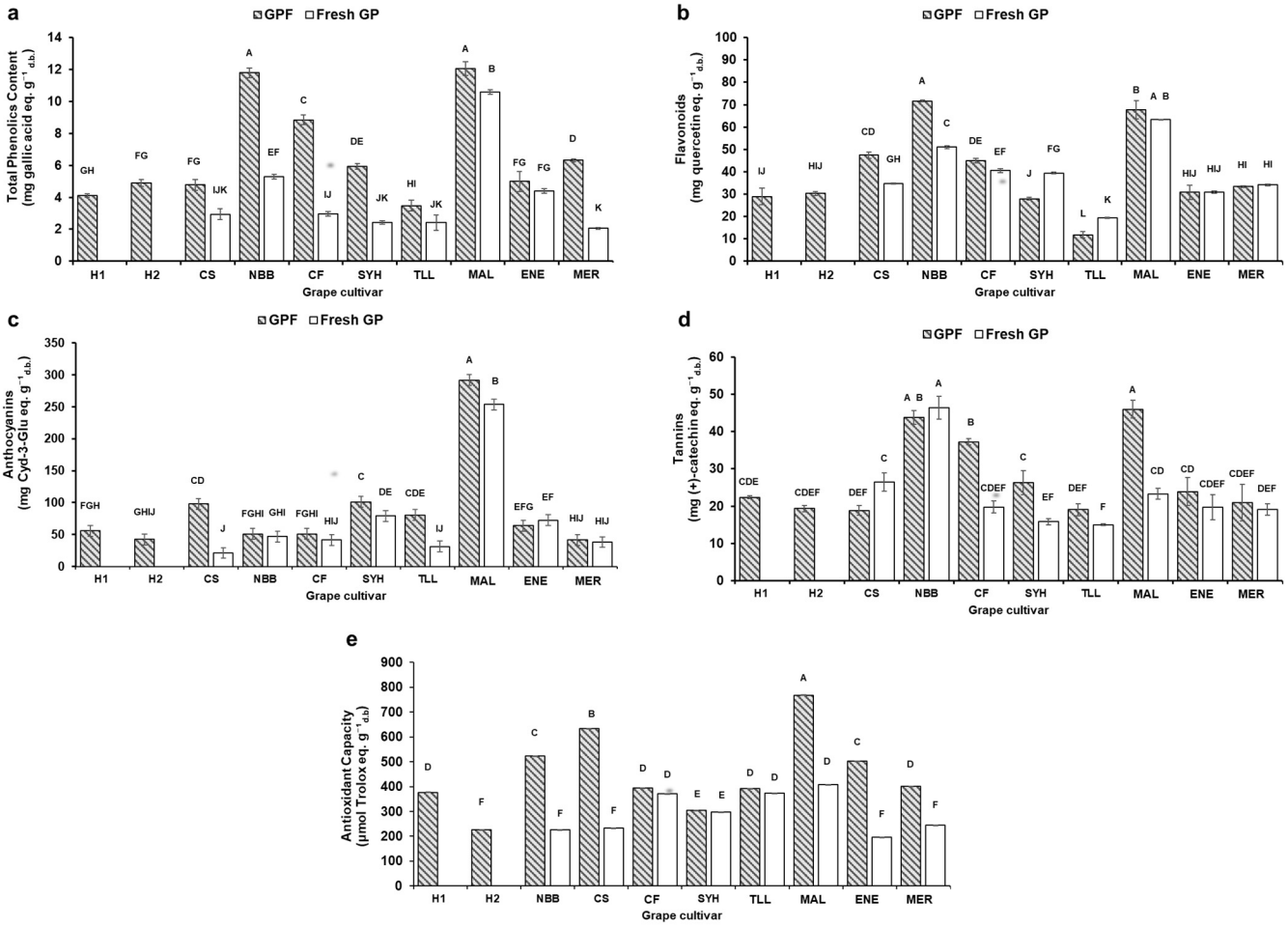


Figure 1. Bioactive compound content and antioxidant capacity of grape pomace flour (GPF) and fresh grape pomace (Fresh GP) from eight grape varieties (CS, Cabernet Sauvignon; NBB, blend Nebbiolo-Sangiovese; CF, Cabernet Franc; SYH, Syrah; TLL, Tempranillo; MAL, Malbec; ENE, Ensamble; MER, Merlot) and two commercial grape pomace flours (H1, Cabernet Sauvignon; H2, Merlot). (**a**) Total phenolics content (mg gallic acid equivalents g^{-1} d.b.); (**b**) Flavonoids (mg quercetin equivalents g^{-1} d.b.); (**c**) Anthocyanins (mg cyanidin-3-glucoside equivalents g^{-1} d.b.); (**d**) Tannins (mg (+)-catechin equivalents g^{-1} d.b.); (**e**) Antioxidant capacity (µmol Trolox equivalents g^{-1} d.b.). Values are expressed as mean ± standard deviation (n = 3 analytical replicates of a single composite sample). Different uppercase letters above bars indicate statistically significant differences (one-way ANOVA, Tukey's test, $p < 0.05$) among all eighteen bars (GPF and Fresh GP, across all varieties and commercial flours) within each panel. A separate two-way ANOVA (cultivar × processing, excluding H1/H2) confirmed a significant cultivar × processing interaction for all five parameters (Appendix A.2, Table A1).

Similarly, the anthocyanin concentration was lower in the GP samples (21.29–253.7 mg cyanidin-3-glucoside eq. g^{-1} d.b.) than in the GPF samples (41.46–291.85 mg cyanidin-3-glucoside eq. g^{-1} d.b.) (Figure 1c). Malbec showed the highest anthocyanin content ($p < 0.05$), both in its GPF (291.85 mg cyd-3-glu eq. g^{-1} d.b.) and fresh GP (253.7 mg cyd-3-glu eq. g^{-1} d.b.), relative to the other composite samples and the commercial flours. This cultivar-dependent response to drying was confirmed by a cultivar × processing interaction ($F_{7,32} = 229.64$, $p < 0.001$; Appendix A.2, Table A1), indicating that the fresh-to-dried difference varied among the composite samples evaluated.

A comparable trend was observed for tannins: the Malbec (45.98 and 23.25 mg catechin eq. g^{-1} d.b., respectively) and Nebbiolo-Sangiovese (43.81 and 46.42 mg catechin eq. g^{-1} d.b., respectively) varieties showed the highest tannin contents (Figure 1d). Notably, Malbec GPF exhibited a 97.8% higher tannin concentration than its fresh GP. Nebbiolo-Sangiovese, on the other hand, showed no significant difference ($p > 0.05$) between its GPF and fresh GP. This contrast was further supported by a cultivar × processing interaction for tannin content ($F_{7,32} = 25.14$, $p < 0.001$; Appendix A.2, Table A1), indicating that the dried-versus-fresh difference was not uniform across the composite samples evaluated; whether this reflects cultivar-intrinsic behavior would require independent biological replication.

Likewise, the antioxidant capacity of the GPFs (304.2–768.3 µmol Trolox eq. g^{-1} d.b.) was higher than that of the fresh GPs (196.6–408 µmol Trolox eq. g^{-1} d.b.) (Figure 1e). Malbec GPF showed the highest antioxidant capacity (768.3 µmol Trolox eq. g^{-1} d.b.), followed by the GPFs made from Cabernet Sauvignon (632.8 µmol Trolox eq. g^{-1} d.b.) and Nebbiolo-Sangiovese (522.4 µmol Trolox eq. g^{-1} d.b.). Antioxidant capacity was higher in the dried than in the fresh composite samples for most cultivars evaluated, but a two-way ANOVA (cultivar × processing state; Appendix A.2, Table A1) confirmed that this effect was not uniform (cultivar: $F_{7,32} = 270.17$, $p < 0.001$; processing: $F_{1,32} = 2616.8$, $p < 0.001$; cultivar × processing interaction: $F_{7,32} = 229.64$, $p < 0.001$), indicating that the magnitude of the fresh-to-dried difference varied among the composite samples evaluated.

On the other hand, although their total flavonoid and phenolic compound contents were similar (Figure 1), commercial GPF H1 (Cabernet Sauvignon) exhibited higher concentrations of anthocyanins and tannins, as well as a greater antioxidant capacity, compared to commercial GPF H2 (Merlot); therefore, H1 was selected as the reference flour in the gummy candy experiments (Section 3.2).

The increase in bioactive compound concentrations observed after drying could be attributed to improved extractability due to cell wall rupture during the drying process, which facilitates the release of intracellular compounds, consistent with findings reported for other grape varieties [30,31]. Drying is a unit operation that significantly affects the integrity of phytochemical-derived bioactive compounds, which are characterized by their thermolabile nature [32,33]. One study showed that conventional drying at temperatures of 100–140 °C caused significant losses of phenolic compounds and antioxidant activity in red grape pomace [34]; in contrast, vacuum drying at 60 °C [35] and 70 °C [18] has been shown to be more effective at preserving bioactive compounds. However, when handling large volumes of residue and implementing the process at an industrial scale, vacuum drying technology could increase the costs of valorizing this residue. In the case of Malbec GP, the post-drying increase in tannins could be attributed to greater extractability, as tissue dehydration could release tannins bound to proteins and cell wall carbohydrates [36,37], which is consistent with the findings reported by Pires et al., who evaluated the effect of drying method on pomace from the *Vitis labrusca* variety Bordo. This study demonstrated a difference in tannin content of up to 176.3% between samples dried by conventional drying versus freeze-drying. The authors determined that forced-air circulation drying at 60 °C for 24 h was more effective for preserving these bioactive compounds [38]. Regarding antioxidant capacity, the changes depend, in part, on the characteristic phenolic profile of each cultivar, since the composition and proportion of anthocyanins, flavan-3-ols, and other phenolic compounds, as well as their interactions with the cell wall, influence their stability under thermal treatment [3].

Beyond the effect of drying, the total phenolic and flavonoid contents and profiles, as well as those of anthocyanins and tannins, are influenced by various environmental factors: harvest time, growing environment, geographic location, grape treatment during the winemaking process, as well as genetic factors of the variety [9,39]. The GP samples

analyzed in this study originated from the same vineyard and were grown and harvested during the same season. Therefore, the observed differences in bioactive compound concentrations among GP samples may reflect interactions between cultivar-specific genetic characteristics and environmental factors influencing secondary metabolism, as well as differences arising from winemaking practices and other sample-specific factors. Because only one composite sample per cultivar was analyzed, the relative contribution of these sources of variation could not be independently assessed or separated.

Specifically, the flavonoid content in Nebbiolo-Sangiovese GPF could be associated with the phenolic profiles of the Nebbiolo and Sangiovese cultivars, which are characterized by a high concentration of proanthocyanidins in the skin and seeds [40]. Meanwhile, the Malbec variety is characterized by significant catechin, quercetin, and dihydroflavonol contents, a phenolic profile reported to be richer than that of other varieties such as Cabernet Sauvignon or Merlot [41]. Conversely, varieties such as Syrah or Merlot have lower concentrations of flavonoids such as catechins and flavonols, since they show lower activity of the flavan-3-ol reductase and leucoanthocyanidin reductase enzymes, which are key to flavonoid metabolism [42]. The Malbec variety has higher concentrations of malvidin, delphinidin, and petunidin, the main pigments in red grapes [43], as well as significantly greater anthocyanin accumulation in its skin compared with Cabernet Sauvignon or Merlot [41], which is consistent with the results obtained in the present study.

In addition to the widely documented antioxidant [44], anti-inflammatory [45], cardiovascular protection [46], and antidiabetic activities of anthocyanins through inhibition of α-glucosidase and α-amylase [47], the presence of anthocyanins in GPF is also relevant from a technological standpoint. Anthocyanins are natural pigments that impart characteristic coloration at certain pH values, a property that could be exploited in products formulated with GPF, given the growing trend toward the use of natural colorants to replace synthetic ones [48]. It is worth noting that, although tannins, particularly proanthocyanidins, are responsible for the well-known antioxidant activity of GP, they can affect sensory attributes related to taste due to their astringent effect, as well as food texture properties, since they form complexes with proteins and carbohydrates. Therefore, the benefits from bioactive compounds' biological activities must be carefully balanced with their negative impact on technological application [38,49].

The antioxidant capacity of GP extracts from different cultivars has been widely documented. Using the ABTS colorimetric assay, an antioxidant capacity of 16.05 µmol Trolox eq. g^{-1} d.b. was measured for the Bogazkere variety [50], while an ensemble of several grape cultivars showed an activity of 8.23 and 8.32 µM Trolox eq. g^{-1} d.b. [51], and Pinot noir pomace extract showed a value of 46 µmol Trolox eq. g^{-1} d.b. The antioxidant capacity of the pomace samples evaluated in this study could be used to protect organic molecules against oxidation, including lipids present in foods; in the formulation of nutraceuticals and dietary supplements for complementary therapy for chronic diseases linked to oxidative stress; and in the formulation of cosmetics and personal care products to prevent aging [52,53].

PCA was performed on the mean values (n = 3) of antioxidant capacity, total phenolics, anthocyanins, flavonoids, and tannins for each variety, for both fresh GP and GPF (Figure 2). Figure 2a shows that, for fresh GP, the first two principal components explained 88.9% of the total variance (PC1 = 54.1% and PC2 = 34.9%). PC1 was mainly associated with total phenolic, flavonoid, and anthocyanin contents, reflecting the polyphenolic richness of GP across the different varieties. PC2, on the other hand, separated antioxidant capacity from tannins and flavonoids. This difference suggests that GPs from varieties with higher flavonoid and tannin contents do not necessarily show higher antioxidant capacities [54]. Along PC1, the Malbec GP sample was separated from the other samples, in association

with its higher anthocyanin and total phenolic contents. Cabernet Sauvignon GP, in turn, was predominantly projected along PC2, suggesting an association with the flavonoid and tannin variables.

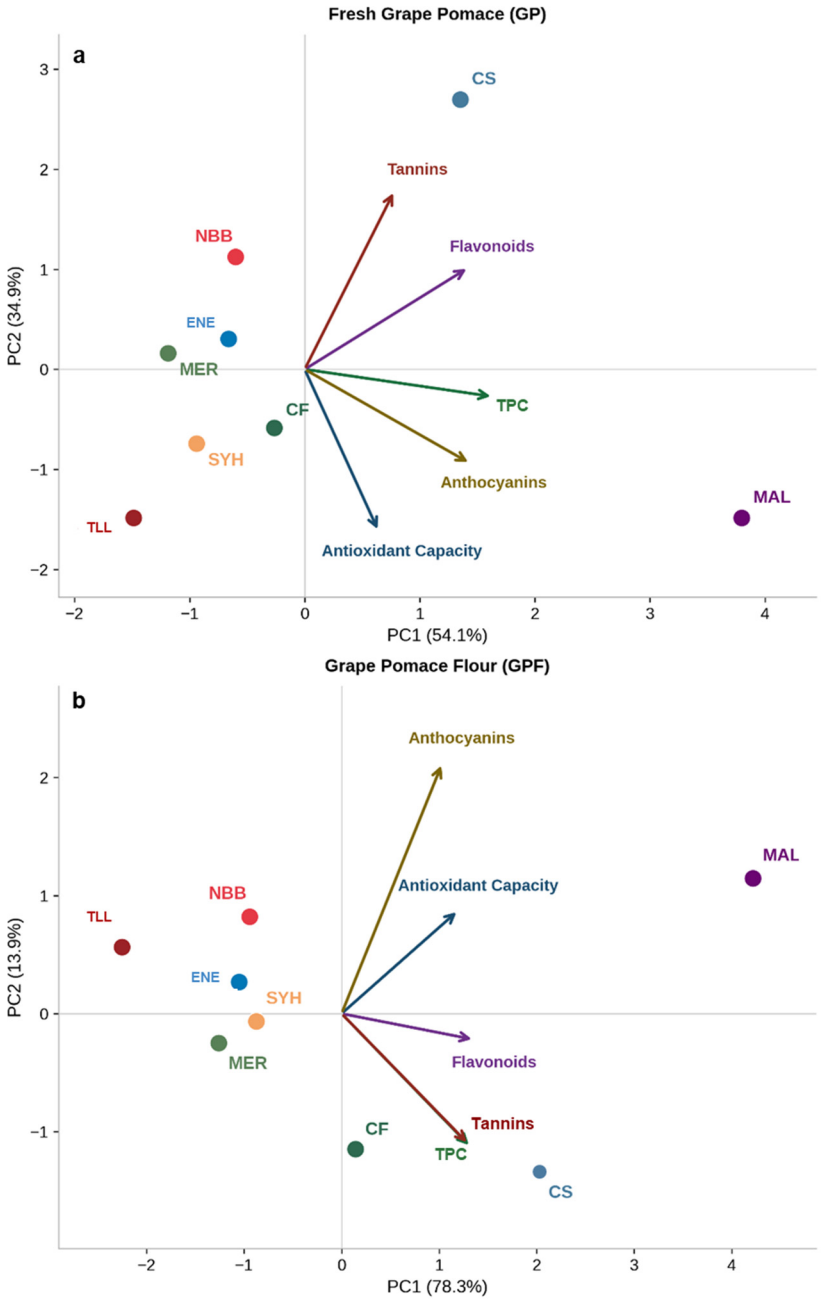


Figure 2. Principal component analysis (PCA) biplots of bioactive compounds and antioxidant capacity in fresh grape pomace (GP, panel (**a**)) and grape pomace flour (GPF, panel (**b**)) from eight grape varieties. Analysis was performed on mean values (n = 3) standardized to unit variance. Colored dots represent variety centroids; arrows indicate the direction and relative contribution of each variable to the principal components. Values in parentheses on each axis indicate the percentage of total variance explained by each component. Varieties: NBB = blend Nebbiolo-Sangiovese, CS = Cabernet Sauvignon, CF = Cabernet Franc, SYH = Syrah, TLL = Tempranillo, MAL = Malbec, ENE = Ensamble, MER = Merlot. TPC = total phenolic content.

Regarding GPF from the different varieties, a clustering pattern different from that of fresh GP was observed (Figure 2b). PC1 explained 78.3% of the variability and was mainly associated with antioxidant capacity, total phenolics, flavonoids, and tannins,

while PC2 (13.9%) was dominated by anthocyanins, showing a distinct behavior from the other parameters. Similar to fresh GP, Malbec GPF was differentiated from the other varieties, mainly due to its association with the variables related to phenolic content and antioxidant capacity.

3.2. Incorporation and Evaluation of Grape Pomace in a Food Matrix

3.2.1. Grape Pomace Flour Proximate Analysis

In addition to its bioactive compound profile, the nutritional composition of an ingredient also affects its feasibility and technological application for incorporation into food matrices. Therefore, a proximate analysis was performed to determine the macronutrient content of grape pomace flour from the Malbec variety (Malbec GPF), which was compared to commercial grape pomace flour H1, in order to further assess its potential as a functional ingredient in a food matrix. Table 1 shows the results for the ash, crude fiber, crude fat, and crude protein contents of Malbec GPF and H1. Crude fiber is of particular interest as it is a component of growing interest in the formulation of functional foods due to its well-recognized health benefits. Both Malbec GPF and H1 showed the highest crude fiber content; this, together with the higher total phenolic, flavonoid, anthocyanin, and tannin contents, and antioxidant capacity previously reported for Malbec GPF (Section 3.1), supports its selection as the candidate flour for the gummy candy application described in Section 3.2.2.

Table 1. Proximate composition of grape pomace flour from Malbec (GPF-Malbec) and commercial grape pomace flour H1 (GPF-H1).

Component (% d.b.)	**GPF-Malbec**	**GPF-H1**
Ash	1.95 ± 0.09	2.19 ± 0.17
Crude fiber	28.71 ± 0.40	31.66 ± 0.53
Crude fat	3.37 ± 0.85	3.25 ± 1.12
Crude protein	3.87 ± 0.12	3.68 ± 0.06
Moisture	5.98 ± 0.16	4.33 ± 0.15
Total carbohydrates *	56.14 ± 0.78	54.89 ± 0.18

Values are expressed as mean ± standard deviation (n = 3). * Determined by difference.

3.2.2. Stability of Color and Bioactive Compounds in GPF-Enriched Gummy Candies During Storage

Color stability is a factor that directly impacts the quality of processed foods since changes in color parameters can reflect physical and chemical changes related to the degradation of chemical compounds, particularly pigments, as well as oxidation–reduction or browning reactions. Although the GPFs made from Malbec and the Nebbiolo-Sangiovese blend showed comparable total phenolic, flavonoid, and tannin contents (Section 3.1), Malbec GPF exhibited the highest anthocyanin content ($p < 0.05$) compared to the other varieties evaluated, including the Nebbiolo-Sangiovese blend; based on this criterion, this variety was selected to evaluate its performance as a natural colorant and source of bioactive compounds in a model food matrix and was compared with commercial flour H1. The color stability of Malbec GPF and H1 was evaluated in gummy candies after storage at 40 °C for 30 days. Figure 3 shows the hues observed for the two batches of gummy candies formulated with Malbec GPF and H1.

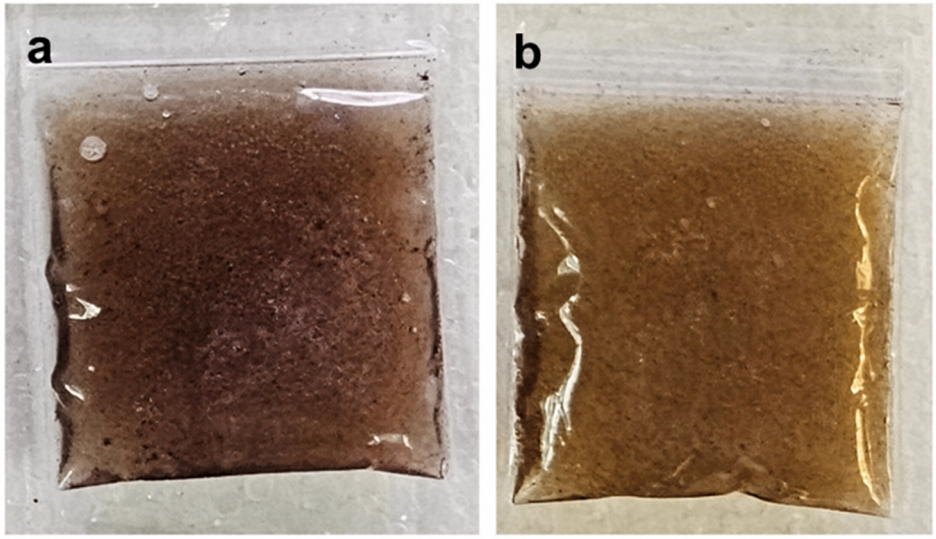


Figure 3. Appearance of gelatin-based gummy candies formulated with grape pomace flour (GPF) on day 0 of storage. (**a**) Gummy candies formulated with GPF from the Malbec variety (GPF-Malbec); (**b**) Gummy candies formulated with commercial grape pomace flour H1 (GPF-H1).

Figure 4 shows the changes in the L^*, a^*, and b^* parameters. At the start of the treatment, the Malbec GPF and H1 gummies showed clearly differentiated colorimetric profiles: Malbec GPF exhibited higher luminosity (L^* = 40.27 vs. 35.72), but lower red intensity (a^* = 25.21 vs. 32.14) and a practically negligible yellow component (b^* = 1.38 vs. 16.54) compared with H1. This reflects the different polyphenolic compositions of each variety, particularly anthocyanins and flavonoids, as discussed in Section 3.1. The color parameters of the GPF-formulated gummies were compared with a control formulation (gummy matrix without GPF) to determine the colorimetric contribution of GPF. On day 0, the control was found to exhibit high luminosity ($L^* \approx$ 66–75) and nearly neutral a^* values (-3.6 to -1.5), demonstrating that the base matrix does not contribute to red pigmentation. During storage, a loss of luminosity (lower L^* values) was observed over time, likely associated with polyphenol oxidation and Maillard-type reactions between reducing sugars and gelatin amino acids [55]. A shift toward red (a^*) was also observed for the candies incorporating Malbec GPF and H1, which could be attributed to the copigmentation effect of anthocyanins [56]. In contrast, the b^* parameter (yellow-blue) showed no changes in the Malbec GPF formulation, but a shift toward blue was observed in the H1 gummies.

To objectively evaluate the color changes in the gummy candies during 30 days of storage, the absolute color difference (ΔE) was calculated. Previous studies have established that ΔE values above 5 indicate color differences that are readily perceptible to an observer, which has become a widely used criterion in food studies [57,58]. In this study, both the Malbec GPF and H1 formulations were found to show a perceptible color difference to the human eye, with ΔE values of 28.50 $\pm$ 2.91 and 21.47 $\pm$ 0.27, respectively, compared with day 0. These results suggest that, although GPF could be used for its color attributes, controlled storage conditions must be maintained to preserve the visual quality of the product.

Table 2 shows that gummy candies formulated with Malbec GPF exhibited higher half-life values, both for total phenolics ($t_{½}$ = 39.6 days) and total anthocyanins ($t_{½}$ = 41.3 days), compared with those formulated with H1 ($t_{½}$ = 19.3 and 26.5 days, respectively). This difference could be attributed to several factors related to the intrinsic composition of GPF and to its behavior within the gummy matrix. Furthermore, it is important to note that the half-life estimates were derived under accelerated storage conditions (40 °C); therefore, they

should not be interpreted as accurate predictions of shelf life under conventional storage conditions, but rather as indicators of relative stability among the formulations evaluated.

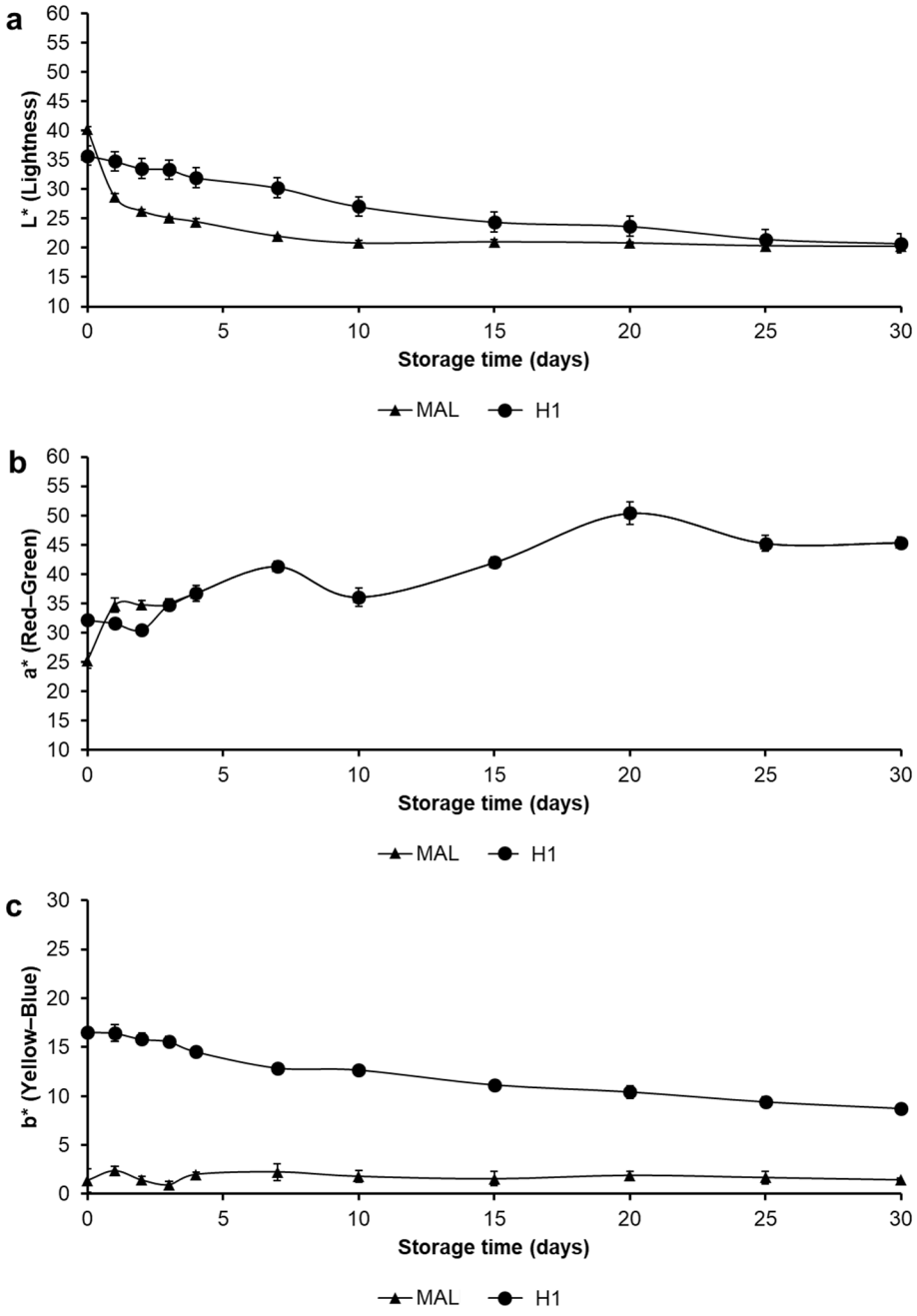


Figure 4. Changes in CIELAB color parameters of gummy candies formulated with grape pomace flour (GPF) from the Malbec variety (MAL) and commercial grape pomace flour H1 (Cabernet Sauvignon) during 30 days of storage at 40 °C. (**a**) *L** (lightness); (**b**) *a** (red–green); (**c**) *b** (yellow–blue). Values are expressed as mean ± standard deviation (n = 3). Sampling days: 0, 1, 2, 3, 4, 7, 10, 15, 20, 25, and 30.

Table 2 shows that the first-order model exhibited a comparatively lower goodness-of-fit for Malbec GPF (R^2 = 0.82 for TPC and 0.81 for TA) than for H1 (R^2 = 0.95 and 0.97, respectively), suggesting that additional factors, such as copigmentation and matrix interaction, could cause the degradation of phenolics and anthocyanins in Malbec to deviate from strict first-order behavior. To further assess this, zero-order and second-order kinetic models were also fitted to the same data (Appendix B, Table A2); none of the alternative

models provided a consistently better fit across both compounds and formulations, so the first-order model was retained since it is widely used to describe phenolic and anthocyanin degradation in food matrices, allowing comparability with previous studies.

Table 2. First-order degradation kinetic parameters of total phenolic content (TPC) and total anthocyanins (TA) in gummy candies formulated with grape pomace flour from Malbec (GPF-Malbec) and commercial flour H1 (GPF-H1) during 30 days of storage at 40 °C.

Parameter	TPC (mg GAE g^{-1})		TA (mg Cyd-3-Glu g^{-1})	
	GPF-Malbec	GPF-H1	GPF-Malbec	GPF-H1
C_0	0.579	0.376	14.734	12.735
R_{30} (%)	61.2	34.3	63.3	46.3
k (d^{-1})	0.018	0.036	0.017	0.026
$t_{½}$ (days)	39.56	19.29	41.25	26.47
R^2	0.82	0.95	0.81	0.97

Note: TPC, total phenolic content; TA, total anthocyanins; C_0, initial concentration; k, degradation rate constant; $t_{½}$, half-life; retention at day 30, R_{30}.

The higher initial total phenolic and anthocyanin contents in Malbec GP favor intermolecular copigmentation interactions. It has been widely documented that anthocyanins associate with other phenolic compounds, forming more stable complexes that are less susceptible to oxidation [56,59]. In addition, the Malbec variety is characterized by a significant amount of malvidin-3-glucoside, one of the most stable anthocyanins due to its methoxyl groups at the 3′ and 5′ positions of the B ring [60]. Moreover, tannins can interact with anthocyanins through copigmentation and condensation phenomena, which can delay their degradation and improve color stability during storage and processing [61]. As discussed in Section 3.1, Malbec GPF showed a higher tannin concentration than H1 (Cabernet Sauvignon), which could be an additional advantage favoring bioactive stability in the gummy matrix.

4. Conclusions

This research evaluated the technical feasibility of valorizing GP from different grape varieties through a simple drying (60 °C, 24 h) and milling strategy. The results showed that the concentrations of bioactive compounds with antioxidant capacity, such as phenolics, flavonoids, anthocyanins, and tannins, are preserved in most cases, and their extractability is even improved after drying, supporting this unit operation as a process for transforming GP into a flour that facilitates its handling and storage. Among the samples analyzed, Malbec GPF showed the highest anthocyanin content and antioxidant capacity, and was therefore selected as a candidate for incorporation into the gummy matrix. The incorporation of Malbec GPF into gummy candies demonstrated its potential as a natural colorant and source of bioactive compounds, owing to its high anthocyanin (natural pigment), fiber, and bioactive compound contents. Malbec GPF was found to exhibit higher bioactive compound retention than H1 under the accelerated storage conditions evaluated (40 °C for 30 days). These findings demonstrate that GP constitutes a residual biomass with high valorization potential within a circular economy framework. It is important to highlight that all grape pomace (GP) samples originated from a single vineyard and a single harvest season, and each variety is represented by a single composite pomace sample that was analyzed in triplicate; therefore, to confirm the generalizability of the differences observed among cultivars, and to establish the economic feasibility, sensory quality, bioaccessibility, and real-time shelf-life performance of GPF as a food ingredient, future studies are needed that include independent biological replicates covering multiple vineyards, harvest seasons,

and geographic regions. Furthermore, this study did not include a safety assessment of the raw material; therefore, analyses for pesticide residues, potentially toxic elements, or contamination by mycotoxins or microorganisms should be conducted before GPF can be used for food-grade applications. Finally, future studies should consider exploring other matrices, both food and non-food, to continue expanding the valorization routes for this residue.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CS	Cabernet Sauvignon
CF	Cabernet Franc
Cyd-3-Glu	Cyanidin-3-glucoside
d.b.	Dry basis
ENE	Ensamble
TPC	Total phenolic content
GAE	Gallic acid equivalents
GP	Grape pomace
GPF	Grape pomace flour
MAL	Malbec
MER	Merlot
NBB	blend Nebbiolo-Sangiovese
PC	Principal component
PCA	Principal component analysis
SYH	Syrah
TLL	Tempranillo
ΔE	Total color difference

Appendix A

Appendix A.1

Appendix A.1.1 Total Phenolics

A 20 µL aliquot of extract was mixed with 1.58 mL of distilled water and 100 µL of Folin–Ciocalteu reagent. After 8.5 min, 300 µL of a 20% $CaCO_3$ solution was added and

thoroughly mixed. The reaction was allowed to proceed for 2 h, after which absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). The calibration curve was generated following the same procedure using a gallic acid standard solution, over a range of 0–0.03 mg mL^{-1} (y = 93.586x − 0.023, R^2 = 0.99). Results were expressed as mg gallic acid equivalents (GAE) g^{-1} d.b. [19].

Appendix A.1.2 Flavonoids

A 100 µL aliquot of extract was mixed with 610 µL of distilled water, followed by the addition of 30 µL of 5% $NaNO_2$. After 5 min, 60 µL of 10% $AlCl_3$ and 200 µL of 1 M NaOH were added. The mixture was thoroughly mixed and centrifuged at 3500 rpm for 5 min. The absorbance of the resulting mixture was read at 510 nm using a UV-Vis spectrophotometer. The calibration curve was generated using quercetin as standard, over a range of 0.5–15 µg mL^{-1} (y = 0.0806x + 0.0739, R^2 = 0.98). Results were expressed as mg quercetin equivalents g^{-1} d.b [20].

Appendix A.1.3 Anthocyanins

Phosphate and acetate buffers were prepared at pH 1 and 4.5, respectively. For the determination, 100 µL of sample were mixed separately with 1000 µL of buffer at pH 1 and pH 4.5. Samples were read at two absorbance wavelengths (520 and 700 nm) using a UV-Vis spectrophotometer for each sample. Anthocyanin content was calculated according to the pH-differential method, using the following equation:

$$Anthocyanin\left(\frac{mg}{L}\right) = \frac{A * MW \times DF * 1000}{\varepsilon * l}$$

where A = $(A_{520} - A_{700})$pH1.0 − $(A_{520} - A_{700})$pH4.5; MW = 449.2 g/mol and ε = 26,900 $L{\cdot}mol^{-1}{\cdot}cm^{-1}$ (molar extinction coefficient for cyanidin-3-glucoside); DF = dilution factor; and l = path length (1 cm).

Results were expressed as mg cyanidin-3-glucoside equivalents g^{-1} d.b. [21].

This method estimates anthocyanin content based on the molar extinction coefficient and molecular weight of cyanidin-3-glucoside; however, in the absence of a calibration curve using the same solvent, these values should be interpreted as estimates rather than absolute quantifications.

Appendix A.1.4 Antioxidant Capacity (ABTS)

The ABTS radical was diluted in phosphate buffer (pH 7.4) until an absorbance of 0.700 ± 0.020 at 734 nm was obtained. A 990 µL aliquot was mixed with 10 µL of the extract to be evaluated, and after 6 min the absorbance was read at 734 nm. The curve was constructed using Trolox as standard (2 mM), over a range of 0–9.0 µg Trolox (y = 10.342x + 0.8792, R^2 = 0.99). Results were expressed as µmol Trolox equivalents g^{-1} d.b. [23].

Appendix A.1.5 Condensed Tannins

A 1% vanillin solution and two HCl solutions (4% and 8%) in methanol were prepared; a 1:1 (v/v) working solution was then prepared using the 1% vanillin solution and 8% HCl. For the assay, 1000 µL of the vanillin-HCl solution were mixed with 200 µL of sample, incubated for 20 min at 30 °C, and absorbance was read at 500 nm using a UV-Vis spectrophotometer. Blanks were also prepared for all samples, following the same procedure but using 4% HCl instead, and their absorbance was subtracted from that of the assay samples. The calibration curve was generated using (+)-catechin as standard, over a range of 0.5–2.0 mg mL^{-1} (y = 0.2452x − 0.0722, R^2: 0.99) Results were expressed as mg (+)-catechin equivalents g^{-1} d.b. [22].

Appendix A.2

Table A1. Two-way ANOVA results (cultivar × processing state) for bioactive compound content in grape pomace and grape pomace flour.

Parameter	F (Cultivar)	*p*	F (Processing)	*p*	F (Cultivar × Processing)	*p*
Antioxidant capacity	270.17	<0.001	2616.80	<0.001	229.64	<0.001
Total phenolics content	394.94	<0.001	26.76	<0.001	70.00	<0.001
Flavonoids	732.98	<0.001	42.33	<0.001	73.73	<0.001
Anthocyanins	656.11	<0.001	105.14	<0.001	19.08	<0.001
Tannins	86.69	<0.001	79.11	<0.001	25.14	<0.001

Note: Two-way ANOVA (cultivar × processing state) performed on the eight in-house grape varieties (H1 and H2 commercial flours excluded, as no fresh-GP counterpart exists for them). df = 7 (cultivar), 1 (processing), 7 (interaction), 32 (residual) in all cases.

Appendix B

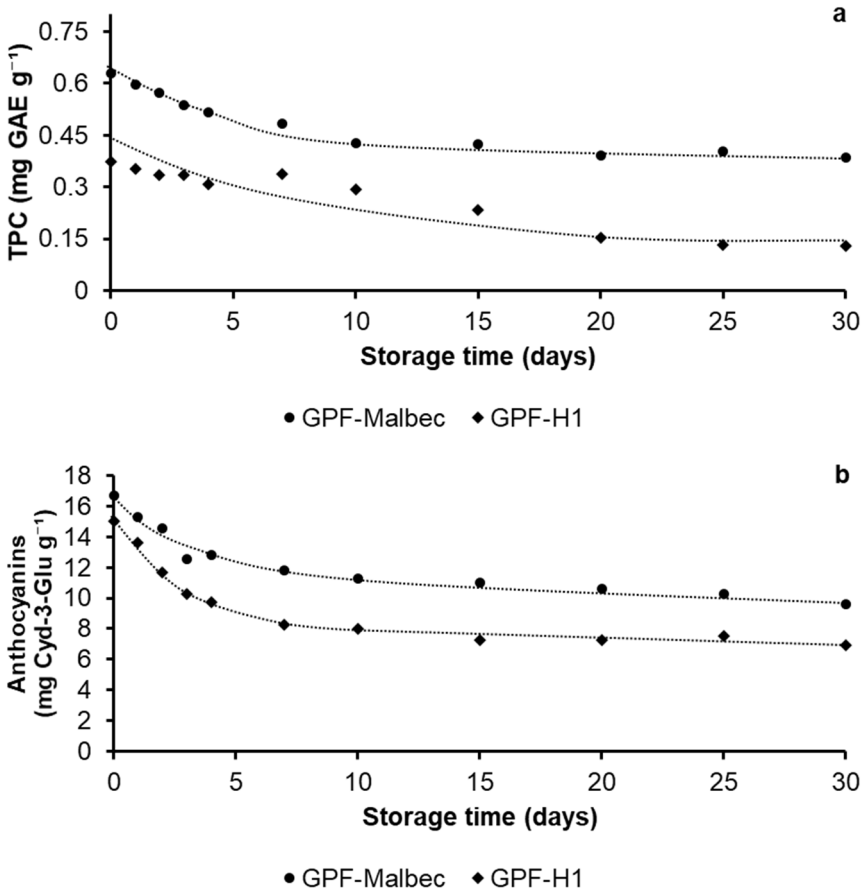


Figure A1. Degradation of (**a**) total phenolic content (TPC) and (**b**) total anthocyanins in GPF-Malbec and GPF-H1 gummy candies during accelerated storage at 40 °C over 30 days. Data are expressed as mean values (n = 3); dotted lines represent the linear trend of each formulation.

Table A2. Comparison of zero-order, first-order, and second-order kinetic models fitted to the degradation of total phenolic content (TPC) and anthocyanins in GPF-Malbec and GPF-H1 gummy candies during accelerated storage (40 °C, 30 days).

Parameter	Sample	Model	Equation	R^2	$t_{½}$ (Days)
TPC	GPF-Malbec	Zero-order	$C = 0.568 - 0.0076t$	0.80	37.48
		First-order	$\ln(C) = -0.547 - 0.0175t$	0.82	39.56
		Second-order	$1/C = 1.757 + 0.0333t$	0.86	52.85
	GPF-H1	Zero-order	$C = 0.364 - 0.0087t$	0.95	20.94
		First-order	$\ln(C) = -0.979 - 0.0359t$	0.95	19.29
		Second-order	$1/C = 2.336 + 0.1836t$	0.93	12.72
TA	GPF-Malbec	Zero-order	$C = 14.441 - 0.1826t$	0.62	39.55
		First-order	$\ln(C) = 2.690 - 0.0168t$	0.81	41.25
		Second-order	$1/C = 0.0703 + 0.00115t$	0.66	61.15
	GPF-H1	Zero-order	$C = 12.296 - 0.2188t$	0.72	28.10
		First-order	$\ln(C) = 2.544 - 0.0262t$	0.97	26.47
		Second-order	$1/C = 0.0814 + 0.0024t$	0.83	33.90

Note: TPC = total phenolic content (mg GAE g^{-1}); TA = Total anthocyanins (mg Cyd-3-Glu g^{-1}).

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